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EXPERIMENTAL SENSITIZATION WITH FOOD DYES AND CROSS-SENSITIZATION TO PARAPHENYLENEDIAMINE*

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CROSS-SENSITIZATION to substances of quinone structure, an allergic response to apparently heterogenous chemical substances, had presented itself, when I[1] first described it in 1928, as a simple, specific sensitization of the skin. Gradually, the problem spread out far beyond its original scope. Today we know that under a common denominator, this cross-sensitization includes a great number of atopic and nonatopic sensitizations as asthma, neurodermatitis, contact dermatitis produced by aromatic amines such as paraphenylenediamine, aminophenol, aniline, sulfonamides, and p-amino-benzoic acid, as well as epidermal sensitizations to various amino-azo dyes used in therapy, in foods, cosmetics, or in the treatment of clothing, leather, papers, wood, etc. (Mayer[2, 3]).

The chemically quite different substances which are bound together in this single biological group have one factor in common which constitutes the directly active principle responsible for the various allergic manifestations. This common factor is an oxidation product formed within the body by a metabolic transformation of the various original allergens. Indeed, by simple oxidation, or in other cases by successive reduction and oxidation, the amines or azodyes are transformed into compounds of quinone structure, the most important of which is quinone diimine (Table I).

Unlike the chemically inert amines and azo dyes, these quinone compounds are highly reactive; they are strong oxidizing agents and readily combine with various amino acids, proteins, and other substances to form complexes of high molecular weight. In this way, quinones of low molecular weight and thus acting only as haptens, are transformed into complete antigens; quinone diimine, bound to protein, constitutes one of the strongest sensitizing agents.

Moreover, these same amines and azo dyes have, in addition to their sensitizing activity, another important property: they produce atypical epithelial proliferations, and certain of these compounds, such as β-naphthylamine and butter yellow, produce malignant growths. As for the allergic activity, the metabolic formation of compounds of quinone structure is responsible for the epithelial action. However, the mechanism of action is not the same in both cases. Quinone diimine acts as an antigen because of its great combining power

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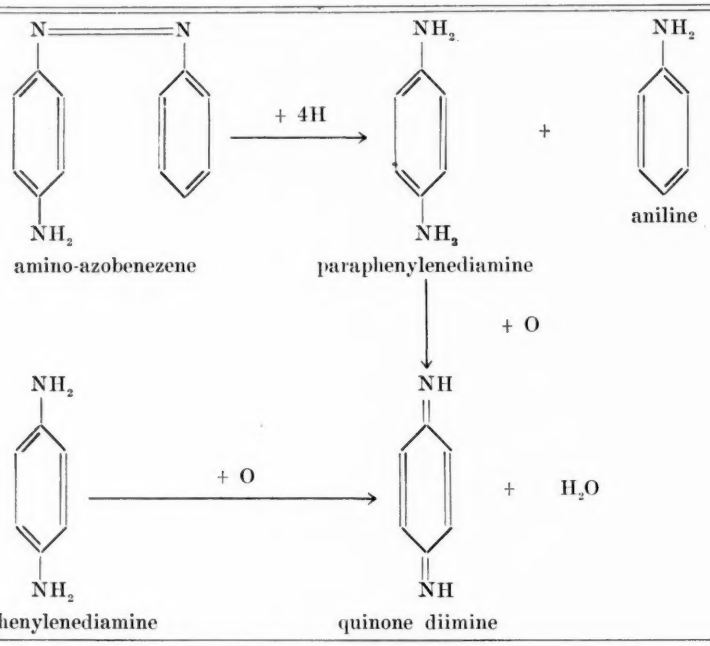
TABLE I. FORMATION OF QUINONE DIIMINE FROM PARAPHENYLENEDIAMINE AND AZO DYES

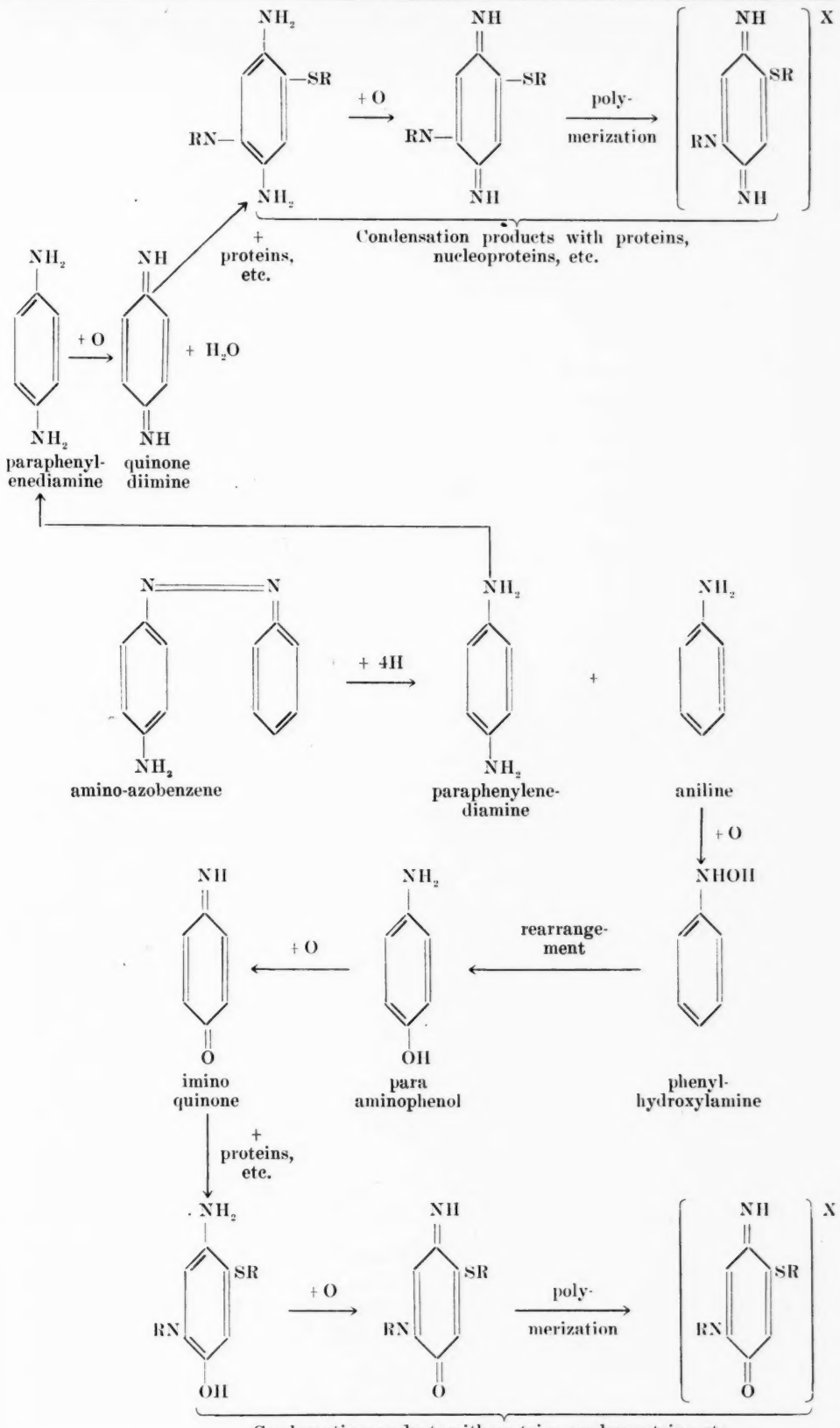


with proteins, and as a stimulant of epithelial proliferation because of its affinity for nucleoproteids (Table II). Quinone diimine is readily attached to cell nuclei and particularly, as I have shown, to the chromosomes and the genes which are composed of nucleoproteids. It is entirely probable that the formation of a quinone diimine nucleoproteid- or a quinone diimine gene-complex is responsible for the proliferative and cancerogenic activity of the various agents in this group of substances (Mayer[4]).

Bearing in mind this background of the cross-sensitization to compounds of quinone structure, we may consider certain recent developments in this field. Allergies produced by substances of this group of aromatic amines and azo dyes are very frequent since the various compounds have wide usage in our everyday lives. Recently a new and important example of cross-sensitization to the compounds of quinone structure has been disclosed by Dobkevitch and Baer,[5] who found that many cases of nylon stocking sensitization are due to a hypersensitivity to the azo dyes employed in the dyeing of the stockings, and that most of these patients are also sensitive to paraphenylenediamine.

As in many other cases of this group of allergies, the nylon stocking sensitizations are characterized by repeated recurrences and sudden activations which occur apparently without any specific cause. Prompted by the thought that these recurrences might be precipitated by the ingestion of certain azo dyes used in food processing, Baer, Leider, and Mayer[6] have been able to show that patients sensitive to paraphenylenediamine and other compounds forming quinone diimine metabolically indeed developed cross-sensitizations to a certain number of azo-food dyes certified by the Food, Drug and Cosmetic Act and widely used in butter, margarine, orange skins, beverages, etc. (Table III).

TABLE II. FORMATION OF COMPLETE ANTIGENS AND NUCLEOPROTEID COMPLEXES FROM AROMATIC AMINES*



*RN— and —SR are amino acids, peptides, proteins, or nucleoproteins attached to various compounds of quinone structure.

TABLE III. PATCH TESTS WITH FOOD DYES IN SUBJECTS HYPERSENSITIVE TO PARAPHENYLENEDIAMINE AND NYLON STOCKINGS (BAER, LEIDER, MAYER)

PATIENT	FD&C RED NO. 32	FD&C YELLOW NO. 3	FD&C YELLOW NO. 5	FD&C YELLOW NO. 6	FD&C ORANGE NO. 2	PARA-PHENYLENE-DIAMINE	NYLON STOCK-INGS
1	+	+++	0	++ to +++	+	++++	+++
2	(+)	+ to ++	0	+ to ++	++ to +++	++++	++++
3	+	++ to +++	0	++ to +++	+ to ++	++++	+++
4	+ to ++	++ to +++	0	++ to +++	(+)	++++	+++
5	+ to ++	0	0	0	0	+ to ++	+++

These results were unexpected since all the dyes had been approved and released for use as food dyes by the Food, Drug and Cosmetic Act. They had been tested by Calvery[7] and found incapable of producing sensitizations in guinea pigs. In reality, however, our results show that they are not as harmless as they were thought to be since they are capable of producing strong allergic reactions in previously sensitized individuals. The fact that substances incapable of sensitizations may nevertheless act as allergic offenders seems quite important from a practical and theoretical standpoint.

EXPERIMENTAL

In order to experimentally distinguish between sensitizing and nonsensitizing amines, we sensitized, in a manner previously described, forty guinea pigs to the following substances: (1) paraphenylenediamine, (2) amino-azobenzene, (3) FD&C Yellow No. 3 (1-phenylazo-2-naphthylamine), and (4) FD&C Yellow No. 6 (Disodium salt of 1-p-sulfophenylazo-2-naphthol-6-sulfonic acid). Twenty-one days after the initiation of the sensitizing treatment the animals of each of the four groups were challenged simultaneously with the aforementioned compounds by intradermal injection and the results noted after twenty-four and forty-eight hours (Table IV).

TABLE IV. BASIC SENSITIZATION

NO. OF ANIMALS	5/10/48 TO 5/19/48 SENSITIZED TO	POSITIVE RESULTS
10	Paraphenylenediamine	100%
10	Amino-azobenzene	10%
10	FD&C Yellow No. 3	10%
10	FD&C Yellow No. 6	0%

RESULTS

After the first sensitizing treatment, during which ten animals of each group were sensitized to a single compound, the cross-sensitivity to all four compounds was examined and the following results were obtained:

All animals pretreated with paraphenylenediamine had become sensitive to this substance; 10 per cent of both the animals pretreated with amino-azobenzene and with FD&C Yellow No. 3 had become sensitive to both of these products, respectively; none of the animals pretreated with FD&C Yellow No. 6 became sensitive to this compound.

With the percentages of sensitizations thus established, each animal was then tested simultaneously with all four different compounds and the results again noted after twenty-four and forty-eight hours (Table V).

TABLE V. CROSS-SENSITIZATION. PERCENTAGE OF POSITIVE REACTIONS TO SKIN TESTS WITH THE FOUR DIFFERENT COMPOUNDS

NO. OF ANIMALS	SENSITIZED TO	POSITIVE CROSS REACTIONS			
		PP	AAB	FD&C NO. 3	FD&C NO. 6
10	PP*	100	90	70	0
10	AAB†	50	10	30	10
10	FD&C No. 3‡	0	0	10	0
10	FD&C No. 6§	0	0	0	0

*PP = Paraphenylenediamine.
†AAB = Amino-azobenzene.
‡FD&C No. 3 = } See Table III.
§FD&C No. 6 = }

The four groups showed the following reactions:

a. Animals sensitized with paraphenylenediamine. All animals gave positive reactions (+ to ++++) with paraphenylenediamine, 90 per cent with amino-azobenzene and 70 per cent with FD&C Yellow No. 3. None reacted with FD&C Yellow No. 6.

b. Of the animals sensitized with amino-azobenzene, only 10 per cent gave positive reactions with the sensitizing agent, but 50 per cent with paraphenylenediamine and 30 per cent with FD&C Yellow No. 3. None reacted with FD&C Yellow No. 6.

c. Of the animals sensitized with FD&C Yellow No. 3, only the sensitizing agent produced hypersensitivitiy in one animal out of ten. No hypersensitivity was produced against the other three substances.

d. In animals sensitized with FD&C Yellow No. 6, no hypersensitivity was produced.

Following this test, all animals were submitted to a new series of three epidermal sensitizing treatments with paraphenylenediamine. Ten days later they were again retested and found to react in the same manner as did the group originally sensitized to paraphenylenediamine (Table VI).

TABLE VI. CROSS-SENSITIZATION ESTABLISHED IN THE SAME ANIMALS OF TABLE V AFTER A CONSECUTIVE SENSITIZATION TO PARAPHENYLENEDIAMINE HAD BEEN PERFORMED

NO. OF ANIMALS	SENSITIZED TO	POSITIVE CROSS REACTIONS			
		PP	AAB	FD&C NO. 3	FD&C NO. 6
10	PP*	100	100	100	0
10	PP	100	50	70	0
10	PP	100	90	90	0
10	PP	60	40	50	0

*PP = Paraphenylenediamine.

This second series of sensitizing treatment, which lasted only three days, was not strong enough to sensitize all animals unless the proceding sensitizing treatment had been made with powerful allergens such as FD&C Yellow No. 3, or, to a lesser degree, amino-azobenzene. Indeed, only 6 positive sensitizations to paraphenylenediamine were obtained in the group of animals first treated with the allergenically inactive azo dye FD&C Yellow No. 6.

DISCUSSION

In these experiments we have tried to establish skin sensitizations with four well-characterized compounds of different antigenic capacity, but nevertheless belonging to the same antigenic group, and then cross-tested all animals with the four allergens. This procedure led to the conclusion that the antigenic group of compounds of quinone structure contains three categories of allergens: first, those, such as paraphenylenediamine, which readily sensitize and also produce in sensitized animals allergic manifestations; second, those, such as FD&C Yellow No. 3, which in themselves are practically unable to produce sensitization but which in animals previously sensitized to a substance of the same antigenic group are able to manifest this sensitivity; and third, those substances, such as FD&C Yellow No. 6, which chemically belong to the group but are, at least in guinea pigs, antigenically inactive and unable to sensitize and produce manifestations in sensitized animals.

The inactivity of the azo dye FD&C No. 6 in guinea pigs is surprising since the same substance produced strong and apparently specific skin reactions in humans sensitive to paraphenylenediamine and nylon stockings (Table II). This is another example of the known fact that guinea pigs do not react to antigenic substances in the same manner as humans. Humans are much more sensitive than the most sensitive guinea pig to certain sensitizing influences, and results obtained from experimental sensitization of guinea pigs do not necessarily indicate what could be expected in humans.

In the experiments reported here, a differentiation has been made between sensitizing and nonsensitizing contact allergens in the case of simple chemical substances. This seems to be a general rule since it is known that certain bacterial products, such as tuberculin or trichophytin similarly contain fractions of different antigenic activity. According to Sulzberger,[8] "Some of the allergenic fractions of tuberculin are antigenic, i.e., can create and produce certain forms of specific sensitivity and specific antibodies; others are partial antigens or haptens, i.e., are capable of eliciting specific reactions, inhibitions, etc., only after the sensitivity has once been established and are not capable of creating a state of sensitivity." I propose to designate as elicitors those azo dyes and other substances which are only able to elicit a present sensitization, as opposed to the substances of the first group, which both sensitize normal individuals and manifest a prevailing sensitization.

As is generally found in allergy, there is no sharp borderline separating the three groups of antigens; we furthermore encounter substances which are weak sensitizers and strong elicitors. Sensitizers and elicitors may be found in other antigenic groups of nonprotein substances, an occurrence which could explain certain cases of allergy of still unknown idiopathic or endogenic nature. Our results also indicate that for a substance to be designated as an innocuous nonallergen, it is not sufficient to prove that it is incapable of producing a sensitization in a normal individual or animal, it only can be so considered if it also fails to produce manifestations in an individual previously sensitized against the more powerful members of its antigenic group.

SUMMARY

1. Certain azo dyes, currently used as food colors incapable of producing sensitizations, are able to manifest a specific sensitization to paraphenylenediamine previously established.

2. The term "elicitors" is proposed for these substances in order to distinguish them from the more powerful sensitizers.

3. It is possible that the cross-sensitizations between paraphenylenediamine and these food dyes has clinical significance.

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DISCUSSION

DR. RUDOLF L. BAER, New York, N. Y.—It gives me particular pleasure to discuss this interesting paper by Dr. Mayer which is based on a contribution which he made in 1928. At that time he showed that, in the skin, azo dyes and paraphenylenediamine are transformed into identical or chemically and immunologically related agents and that this transformation is the basis of the cross-sensitization occurring clinically among these compounds.

Dr. Mayer in his presentation today has stressed one point, which has been known for many years, but which is not sufficiently considered by many clinicians: through cross-sensitization a patient can become allergic to many substances to which he has actually, never previously been exposed. Dr. Mayer calls these substances "elicitors." I prefer to call them "secondary allergens" and to speak of the original sensitizing allergen as the "primary allergen."

As far as the practical importance is concerned of cross-sensitizations among substances containing aromatic amines as part of their molecule, I would like to call attention to the fact that these cross-sensitizations in addition to the azo dyes and paraphenylenediamine may involve such a wide range of substances as many local anesthetics, some sulfonamides, sun-filters such as glycerol paraaminobenzoate and picric acid, and other aromatic nitro compounds.

The opportunities for exposure to azo dyes alone are so tremendous that it is hard to conceive how anybody could escape them. One might say that every time we eat or drink something we are likely to be exposed to azo dyes certified for foods, drugs, and cosmetics, e.g., in confectionery, oleomargarine, butter, sausage casings, beverages, baked goods, maraschino cherries. To this must be added innumerable exposures to azo dyes which are certified only for drugs and cosmetics or for external use in drugs and cosmetics. One might also say that every time we get dressed we expose ourselves to azo dyes as they make up about 50 per cent of the dyes used in textiles.

There can be little doubt that azo dyes by external contact with the skin in some cases causes eczematous allergic dermatitis on the basis of cross-sensitization after primary sensitization to paraphenylenediamine. However, an as yet unanswered question is whether the ingestion of these azo dyes causes eruptions in susceptible subjects. For example, will a beverage which contains one of the certified food azo dyes produce clinical signs or symptoms in persons who are allergic to paraphenylenediamine? This question is of the utmost importance because in the answer to it may be the explanation for the chronicity and tendency to recurrences in some eczematous eruptions. Unfortunately, the answer is not yet available.

I can say that a few preliminary tests done by Dr. Leider and myself have not led to positive results. These tests consisted of feeding paraphenylenediamine-hypersensitive women up to 50 mg. of one of the azo dyes to which cross-sensitization had been demonstrated in these women. However, I want to repeat that these negative results with feeding are entirely preliminary, the number of tests is as yet inadequate and the quantities of azo dyes fed to these patients may have been too small.

I would like to stress, in case somebody tries to do work with azo dyes, that some of these dyes are primary irritants when applied to the skin. However, Dr. Mayer, Dr. Leider, and I were able to conclude from the significantly *stronger* reactions in paraphenylenediamine-sensitive subjects as compared with normal control subjects that a sensitization to azo dyes probably was present in the paraphenylenediamine-sensitive subjects.

The results of the tests in Dr. Mayer's experiment in animals and those jointly obtained by us in human beings indicate how difficult it is to predict, through the present customary tests, whether a substance is likely to cause dermatitis among consumers. The tests, as required by various governmental agencies, are likely to give one a rough estimate of the sensitizing potential. However, they are entirely inadequate to foretell whether a substance is capable of *eliciting* dermatitis even though this substance may not be a sensitizing agent.

DR. R. L. MAYER.—Dr. Baer's interesting remarks on the problem of "elicitor" substances call for some clarification because I wish to prevent any misinterpretation. In each particular case of allergic sensitization we may find one primary and many secondary allergens. According to Dr. Baer, the substances which I have designated as "elicitors" are identical to those usually referred to as "secondary allergens." I believe that a basic difference exists between secondary allergens and elicitors.

Secondary allergens are all those allergens which give positive cross-sensitization in those cases of allergy which were primarily brought about by another but related allergen acting as the sensitizer. There can be no doubt that one finds all sorts of allergens among the secondary allergens, many of which are the strongest sensitizers known and which are as strong or even stronger sensitizers than the primary allergen. Others, however, are devoid of sensitizing power; it is these substances which I call elicitors.

Whether an allergen is a primary or secondary one is a problem of chance; it is sometimes purely accidental when an individual comes in contact with a sensitizing substance. Furthermore, it is often almost impossible to decide which substance is the primary or secondary allergen. Indeed, one may correctly call a substance a secondary allergen only when it has been proved that the cross-sensitization has not been produced by itself although it produces a positive skin reaction. In Meltzer and Baer's example of a cross-sensitization,* the primary allergen was perhaps benzocaine and all the other substances were secondary allergens. But this may be incorrect in view of the patient's previous attacks of atopic dermatitis of undiagnosed etiology. As far as the many cases of nylon stocking sensitizations are concerned where the patients were sensitive to nylons and to paraphenylenediamine, their cross-sensitivity was first known to doctors through their nylon sensitization, but it is very possible that some or all of them had been sensitized much earlier in their lives to paraphenylenediamine primarily. In these cases the nylon stocking dermatitis would be produced by a secondary allergen, although the doctor or even the patients themselves do not recall this first sensitization.

Contrary to this changing aspect of primary and secondary allergens, dependent upon time-factors only, the faculty of being a sensitizer or only an elicitor has nothing to do with time or chance. To be either sensitizers or elicitors constitutes an inherent, intrinsic property of any drug.

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FIXED ANAPHYLACTIC SENSITIZATION*

Bromsulphalein Studies

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THE phenomenon of fixed tissue sensitivity to drugs is by no means new, the term "fixed" referring not to the duration or persistence of the lesion, nor to the residual pigmentation, but solely to the fact that the location of the lesion on the body is "fixed"; that is, a previously affected area can be activated by re-exposure to the drug or other excitant. The morphology of such eruptions is rather characteristic and the condition is usually associated with drugs such as antipyrine, acetphenetidin and phenolphthalein. However, Abramowitz and Noun[1] and Chargin and Leifer[2] have reported cases due to sulfonamides, salicylates, and barbiturates. Furthermore, Abramowitz and Russo[3] have suggested that agents other than drugs can produce the same clinical picture, for example, foods.

Various investigators (Naegeli, Quervain, and Stalder,[4] Knowles, Decker, and Kandle,[5] Urbach and Sidaravicius,[6] Wise and Sulzberger[7]) have studied fixed drug eruptions by the autotransplantation method in an endeavor to determine whether the sensitivity could be transferred from the original site to a new skin site and conversely, whether normal skin when placed in an area of previous reaction would become reactive. The results in general were conflicting. Direct skin testing has also been inconclusive. Urbach[8] and Loveman[9] reported that while epidermal and intradermal tests with the drugs failed to elicit reactions in normal skin sites in their patients, the reactions were positive when performed on previously affected areas. Most often, however, the sensitivity is demonstrated only on ingestion of the offending drug.

Obermayer and Pick[10] produced antigens specific to the chemical part of a protein conjugate and Landsteiner[11] demonstrated the anaphylactogenic properties of proteins conjugated with drugs. Assuming the possibility of conjugation within the body, their findings suggest that the introduction of drugs could engender an anaphylactic type of sensitization. The syndrome of fixed tissue sensitivity, however, does not present a uniform pattern of sensitization. Immediate wheal type of reactions to drugs, especially penicillin,[12] is as a rule associated with generalized, not fixed, sensitivity. On the other hand, fixed angioneurotic edema, such as seen after aspirin medication, fails as a rule to give positive skin reactions.

The case here reported describes a specifically acquired fixed tissue reaction of the immediate wheal type, in that the sensitivity was produced by a sub-

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cutaneous injection of bromsulphalein, was sharply limited to the area of original sensitization, and gave immediate wheal reactions to intradermal injections of dilutions of the sensitizing substance. Since the introduction in 1924 of bromsulphalein as a means of testing liver function, local and systemic toxic reactions have been noted and recorded in the literature and are commonly recognized. However, a review of the literature has failed to reveal any reports of true sensitization to bromsulphalein. Chambers and Moister[13] recently reported a shock reaction after rapid intravenous injection of bromsulphalein into an allergic individual but the reaction could not be interpreted as being of the anaphylactic type.

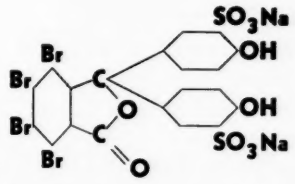
CASE REPORT

K. K., a 53-year-old white man, with advanced Laennec's cirrhosis, had been under study in the Illinois Research Hospital for 119 days. There was no past history of allergic reactions with the exception of a suspected wool sensitivity. During his stay in the hospital, bromsulphalein clearance tests (5 mg. per kilogram), were done at weekly or biweekly intervals. No reactions or difficulties were encountered. On the one hundred twentieth hospital day, about 0.25 c.c. of bromsulphalein solution was inadvertently injected paravascularly into the subcutaneous tissues of the right forearm. The patient complained of pain in the area and within twenty-four hours developed a kidney-shaped, painful, circumscribed inflammatory response, approximately 10 × 15 cm. in size, in the subcutaneous tissues of the flexor surface of the right forearm. Elevation, immobilization, and the local application of heat produced rapid improvement and complete subsidence of the inflammation within four days. One month later, 7.1 c.c. of bromsulphalein solution was injected fairly rapidly into the median antecubital vein on the opposite (left) forearm. Thirty to sixty seconds after the injection, the patient experienced nausea, retching, weakness, and severe abdominal cramps. He first flushed and then became pale, but there was no sweating. He had a tachycardia of 110 beats per minute and the radial pulse was weak. During the next sixty seconds, the flexor surface of the right forearm and the antecubital space became reddened, painful, and swollen. The sharply outlined elevated, inflammatory area was of exactly the same size and position as the first local response, which had occurred a month previously, and the pain was of equal intensity. At the same time as the reaction occurred on the right arm, a group of urticarial wheals, ranging in size from 1 to 6 cm. in diameter, appeared on the patient's abdomen, back, buttocks, and extremities. Lesions did not appear on the head or neck. The patient had moderate conjunctivitis, but no engorgement of the nasal mucosa. No evidence of asthma was found on physical examination, nor did any diarrhea occur. Epinephrine, 0.3 c.c. of 1:1,000 solution, was given intramuscularly and 50 mg. of Benadryl, orally. The systemic manifestations and the intense pruritus accompanying the urticaria were relieved within thirty to forty minutes. There was no residual pigmentation, nor any outward sign of difference between the reactive area and the same area on the opposite arm.

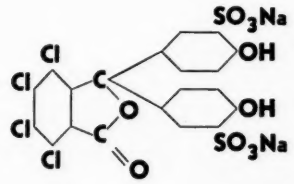
Studies.—On referral to the Allergy Clinic several months later, the area of reaction on the right forearm was tested by the intradermal injection of 0.02 c.c. of a 1:100 dilution of bromsulphalein solution. Within a few minutes an urticarial-like wheal, about 2 cm. in diameter, surrounded by an area of hyperemia developed at the site of injection. A control intradermal injection on the opposite (left) forearm gave no response. The right arm and forearm were then tested at various sites and it was found that the immediate wheal reaction could be elicited only within the area of the inflammatory response to the original paravascular injection, and that there was a diminishing response as the borders of the area were approached. Various dilutions of bromsulphalein were used for intradermal testing and it was observed that wheals could be obtained with dilutions up to 1:1,000 and erythema with dilutions up to 1:100,000. Inhibition of the immediate wheal reaction could be accomplished by administering 100 mg. of Pyribenzamine one hour before testing. The question was considered whether nonspecific factors, such as edema and inflammation, had rendered the area

of extravasation hyperreactive. Accordingly, both forearms were tested with serial dilutions of histamine phosphate in buffered saline and it was found that the response was the same on both forearms. Inasmuch as the patient had given a history of irritation from wool blankets, the skin of the back was tested with wool extract, dilution of 1:1,000, and a positive reaction obtained. Intradermal tests with dilutions of wool extract gave the same size response on both forearms.

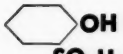
Other means of eliciting a response to bromsulphalein were tried. Patch tests with 5 per cent bromsulphalein solution gave negative results on both forearms. One-half ampule of bromsulphalein solution was given by mouth with no reaction, either systemic or local. Finally, 2 c.c. of a 1:10 dilution of bromsulphalein solution was injected intravenously into the median antecubital vein of the left forearm. No systemic reaction occurred, but seven minutes after the injection, a wheal appeared on the right (sensitized) forearm at the site of a healed cantharidin blister.



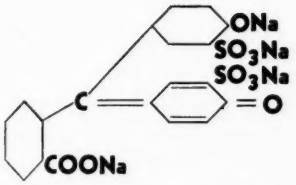
1. Di-sodium tetrabromophenolphthaleinsulfonate (Bromsulphalein)



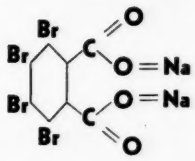
2. Di-sodium tetrachlorophenolphthaleinsulfonate



4. o-Phenolsulfonic acid



3. Sodium phenolphthaleinsulfonate



5. Di-sodium tetrabromophthalate

Fig. 1.

No attempt was made to transfer the hypersensitiveness through the autotransplantation method, but repeated attempts at passive transfer by the Prausnitz-Küstner technique were unsuccessful.

A marked tissue eosinophilia (30 per cent) was found over the area of sensitization, while tissue fluid from the remaining skin was practically free from eosinophiles.

Bromsulphalein chemically is di-sodium tetrabromophenolphthaleinsulfonate (Fig. 1). Its tetrachloro analogue (di-sodium tetrachlorophenolphthaleinsulfonate) elicited exactly the same responses in the sensitized area as the bromsulphalein. However, a halogen-free analogue

(sodium phenolphthaleinsulfonate) gave negative results on testing. Two compounds representing parts of the bromsulphalein molecule, namely, phenolsulfonic acid and di-sodium tetrabromophthalate, also failed to elicit reactions.

Phenolphthalein, phenolsulfonphthalein, and tetraiodophenolphthalein were also employed for testing; the first, which is insoluble in water, by the scratch method and the latter two by the intradermal method. All gave negative results.

DISCUSSION

Bromsulphalein has been employed widely as a test of liver function. Hitherto, there have not appeared any reports of sensitization following the use of the dye. To a certain degree this could have been expected, as the material is injected intravenously and such a route of administration is notoriously a poor one in engendering experimental anaphylactic sensitization. Morey, Gabuzda, and Scudamore,[14] however, report two cases in which there occurred severe pain along the course of the vein and the appearance of ten to fifteen urticarial wheals about 1 cm. in diameter around the site of the needle puncture. Both patients had had several previous intravenous injections of the dye into the same vein and at the same site and it is suggested that some of the material was spilled extravascularly and sensitized the surrounding tissues.

In the case here reported it would appear that a number of previous injections of bromsulphalein were tolerated by the patient without any evidence of sensitization. However, following a paravascular (subcutaneous) injection, a local inflammation developed which soon subsided on simple management with no outward sign of any change in the patient's sensitivity. When given bromsulphalein solution intravenously one month later, he responded with a generalized reaction indicative of an antigen-antibody reaction with release of histamine. The most interesting observation, however, was the flare-up at the site of the previous inflammation and the prompt response, both systemically and locally, to epinephrine and Benadryl. Subsequent intradermal testing revealed an immediate wheal reaction obtained only within the area of initial inflammation, indicating that sensitization was fixed in this area. It is felt that the bilaterally equal responses to histamine and wool extract excluded the possibility of an increased nonspecific hyperreactivity. The inhibition of the specific intradermal tests by the prior administration of Pyribenzamine certainly suggests that the immediate wheal type of reaction was due to liberation of histamine and that the shock symptoms observed on intravenous injection following the original sensitization were on the basis of histamine release. It is assumed that the bromsulphalein acted as a hapten and combined with body protein to form a complete antigen.

The exact portion of the bromsulphalein molecule responsible for the development of a fixed anaphylactic sensitization could not be determined with the compounds available. Both the original compound and its tetrachloro analogue gave positive reactions, while the halogen-free analogue of bromsulphalein gave negative reactions. As previously stated, neither di-sodium tetrabromophthalate nor phenolsulfonic acid were sufficient to evoke the reaction.

The intravenous injection of a small amount of bromsulphalein failed to produce the type of anaphylactic response noted originally, but this lack of

reaction might be explained on a quantitative basis. A wheal did appear at a localized, previously altered site within the sensitized area. Furthermore, there was no appreciable diminution in the dose of bromsulphalein required to evoke an immediate wheal response; in other words, the many intradermal skin tests and the intravenous injection did not desensitize the area of fixed sensitization.

SUMMARY

1. A case of fixed tissue sensitivity of the anaphylactic (immediate wheal) type is described.

2. Bromsulphalein injected paravascularly (subcutaneously) resulted in an area of inflammation.

3. Intravenous injection of bromsulphalein one month after the paravascular injection produced an immediate flare-up at the site of the previous inflammatory reaction, a generalized urticaria, and systemic symptoms resembling anaphylactic shock. Both local and systemic symptoms responded promptly to epinephrine and Benadryl.

4. The area of extravasation was found to be sensitive to bromsulphalein and a very closely related halogen analogue, giving immediate wheal reactions on intradermal testing. The whealing response could be inhibited by the prior administration of Pyribenzamine.

5. Studies to correlate chemical structure and skin reactivity were attempted. The tetrachloro analogue of bromsulphalein yielded exactly the same reactions, but a halogen-free analogue did not react. Compounds representing component parts of the bromsulphalein molecule also failed to elicit reactions.

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THE CHEMICAL ISOLATION AND BIOLOGIC ASSAY OF EXTRACELLULAR ANTIGENIC FRACTIONS FROM PATHOGENIC FUNGI*†

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THE nature and evolution of an infectious disease are conditioned by the primary quality of the infecting agent and of the infected host, and by the secondary changes in both that are due to mutual influences. The complete knowledge of all of these factors is necessary for a profound insight into the true nature of the infectious process. The pathogenesis of bacterial diseases has received more careful scrutiny than the mycoses. The very nature of the mycotic infection to proceed slowly at first and then become accelerated suggests that the causative fungi at first rely on dead or injured tissue to grow, that they have slight inherent invasive power, and that they are able to spread only after some change has taken place in themselves or in their environment. There has not been any evidence to date that they increase in virulence with the progress of the infection. It is reasonable to assume that during the course of the mycotic infection the tissues of the host become altered.

The tissues of the host might be altered by endotoxins liberated by the fungi or by the fact that the fungi or their breakdown products sensitize the neighboring cells thus making them susceptible to injury. There is no evidence to support the theory that pathogenic fungi produce endotoxins, nor is there any decisive evidence to date that tissue damage in fungous infections is due to the state of hypersensitivity. Technical difficulties prevent the demonstration of endotoxins from pathogenic fungi and very little work has been done on the mycoses with the thought in mind of explaining tissue damage on the basis of hypersensitivity.

To date the application of immunologic principles to the diagnosis and treatment of mycoses has been generally unsatisfactory. Most attempts to carry out agglutination reactions have been unsuccessful. Very little study of precipitins has been conducted. The complement fixation reaction has given useful information only in coccidioidomycosis. The cutaneous skin reactions to broth filtrates of various fungi, such as trichophytin, Oidiomycin, coccidioidin, histoplasmin, and blastomycin, have been studied. For the most part, reactions obtained from these antigens are not completely reliable in regard to their specificity. Coccidioidin is fairly specific; Oidiomycin gives many nonspecific reactions; Histoplasmin and blastomycin give cross reactions and even though the incidence of positive reactions to these antigens in man are surprisingly high it has been impossible to evaluate the clinical and epidemiological significance. The importance of a positive trichophytin reaction, in view of the frequency of dermatomycosis of the skin, is always to be questioned.

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Coccidioidin, histoplasmin, and blastomycin are usually and best prepared by growing *Coccidioides immitis, Histoplasma capsulatum,* and *Blastomyces dermatitidis,* respectively, on a synthetic medium employing asparagine as a source of nitrogen. The medium is similar to that used in making tuberculin. Oidiomycin is not prepared from a synthetic medium and trichophytin has been manufactured in various ways, employing at times one or a combination of different species of the genus Trichophyton.

The greatest amount of investigation on antigenic fractions derived from fungi has been directed toward various species of the genera Trichophyton and Candida. The majority of the work on the Trichophyton fungi and their antigens has come from Jadassohn, Bloch, and their co-workers. The type of trichophytin antigen most employed in their work was prepared according to the technique of Bloch, Labouchere, and Schaaf,[1] and represents an evaporated broth filtrate plus cell sap from mycelium. A summary of the extensive research on this subject by Jadassohn and his associates has been ably epitomized by Sulzberger.[2] The active principle of trichophytin prepared according to Bloch's[1] method was a nitrogen containing polysaccaride, which has been referred to by Sulzberger[2] as "desiccated trichophytin." Jadassohn[2] prepared trichophytin by this technique from *Achorion quinckeanum (Trichophyton quinckeanum),* Trichophyton Gypseum *(Trichophyton mentagrophytes),* Epidermophyton Kaufman-Wolf *(Epidermophyton floccosum),* and *Achorion Schoenleini (Trichophyton Schoenleini)* and concluded that these four pathogenic fungi contained one or more antigens in common. However, he was not able to isolate from either the fungus cells or their culture medium an antigenic substance that was in itself specific and characteristic only for the fungus from which it was derived.

Soluble substances with characteristics of polysaccarides have been isolated from the cells of various species of Monilia (Candida).[3] These polysaccarides, however, lack species specificity when employed as antigens in precipitin reactions. Furthermore, polysaccarides prepared by the method described by Kesten and his associates[3] will not actively sensitize guinea pigs.[4]

Coccidioidin, a broth filtrate of *C. immitis* grown on synthetic asparagine medium, has been studied extensively and recently Hassid, Baker, and McCready[5] isolated a polysaccaride from the culture filtrate that possessed interesting immunologic properties.

It has been our hope that by broadening the knowledge of culture filtrate antigens of some of the pathogenic fungi that we might be able to improve the reliability of immunologic methods of diagnosis. Furthermore, it would be of theoretical and practical interest to isolate a purified substance that would act with the specificity of a single antigen for the fungus from which it was isolated.

METHODS AND MATERIALS

I. *Source of Fungus Strains.—Tr. mentagrophytes* No. 640 was supplied to us by Dr. Chester Emmons of the National Institute of Health, Bethesda, Maryland. *T. mentagrophytes* No. 599 was supplied to us by Dr. Rhoda H. Benham, Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York. *Trichophyton rubrum* was isolated by us from a patient

with dermatomycosis of the feet. *Candida albicans* was isolated by us from a patient with cutaneous Candidiasis (moniliasis). *H. capsulatum* and *B. dermatitidis* were obtained from the American Type Culture Collection, Georgetown University, Washington, D. C.

II. *Composition of Media.—T. mentagrophytes* and *T. rubrum* were grown on a medium that had the following composition: Casamino Acids (Difco), 5.0 Gm.; dextrose, 10.0 Gm.; K_2HPO_4, 0.75 Gm.; NaCl, 1.0 Gm.; $MgSO_4 \cdot 7H_2O$, 1.5 Gm.; ferric citrate, 0.02 Gm.; and distilled water to make 1 liter.

B. dermatitidis and *H. capsulatum* were grown on a medium that had the following composition: Casamino Acids, 7.5 Gm.; dextrose, 15 Gm.; K_2HPO_4, 1.5 Gm.; $CaCl_2$, 0.1 Gm.; ferric citrate, 0.05 Gm.; and distilled water to make 1 liter. In addition, the medium for *B. dermatitidis* contained 10 mg. p-aminobenzoic acid per liter, and that for *H. capsulatum* contained 2 ml. Lederle Vitamin B Complex* per liter.

C. albicans was grown on a completely synthetic medium which was prepared in two parts, as follows: Part A, 0.4 Gm. glycine, 0.6 Gm. l-leucine, 0.8 Gm. l-glutamic acid, 50 mg. l-arginine · HCl 50 mg. l-histidine · HCl · H_2O, 50 mg. l-tyrosine, 50 mg. l-cystine, 5 Gm. K_2HPO_4, 0.5 Gm. $MgSO_4 \cdot 7H_2O$, NH_4Cl, 0.5 Gm. NaCl, 0.1 Gm. $CaCl_2$, 25 mg. ferric citrate, 5 mg. $ZnSO_4 \cdot 7H_2O$, 5 mg. cupric acetate, 25 ml. N/20 HCl (in which the tyrosine and cystine were first dissolved), 25 ml. N/20 NaOH, distilled water to make 750 milliliters; Part B, 15 Gm. dextrose, 25 mg. Ca-d-pantothenate, 25 mg. inositol, 25 mg. nicotinamide, 25 mg. pyridoxine · HCl, 25 mg. p-aminobenzoic acid, 25 mg. thiamine · HCl, 10 mg. riboflavin, 20 mg. biotin, distilled water to make 250 milliliters. The two parts were autoclaved separately and then mixed with sterile precautions in the ratio of three volumes A to one volume B.

III. *Cultivation of the Fungi.*—The medium was usually prepared in a 5-liter batch and dispensed in 100-ml. amounts in 500-ml. Erlenmeyer flasks. After autoclaving, the medium in each flask was inoculated with a small bit of growth from a Sabouraud's agar culture of one of the fungi. The flasks were kept in an incubator at 30° centigrade. The growth period for *T. mentagrophytes* and *T. rubrum* was three weeks; for *B. dermatitidis, H. capsulatum,* and *C. albicans,* five weeks. The flasks containing *C. albicans,* which formed a sedimentary growth consisting of single cells, were shaken momentarily by hand twice a day.

IV. *Isolation of Fungous Products From Culture Filtrates.*—At the end of the cultivation period the fungous growth was removed by filtration. The combined filtrates were sterilized by passage through a Seitz filter.

The sterile filtrate was concentrated by ultrafiltration through guncotton membranes deposited on alundum shells, according to the method used by Seibert[6] in the preparation of purified tuberculin protein. Our membranes were prepared from 12 per cent by weight solutions of guncotton in glacial acetic acid.

*Containing, per milliliter, 10 mg. thiamine·HCl, 0.5 mg. riboflavin, 10 mg. niacinamide, 1.0 mg. pyridoxine·HCl, 5 mg. pantothenic acid, and vitamin extracts derived from 50 Gm. of liver.

The ultrafiltration process was carried out at 5° C., a negative pressure of approximately 28 mm. Hg being used to draw off the ultrafiltration which was discarded. With four ultrafilters in use, about two days were required to achieve a 50-fold concentration of 3.5 to 4 liters of culture filtrate. No preservative was added to the culture filtrate during the ultrafiltration process.

In the case of *T. mentagrophytes* and *T. rubrum,* solid $(NH_4)_2SO_4$ was added to the concentrated culture filtrate in the ratio of 6.5 Gm. per 10 milliliters. The resulting precipitate was isolated by centrifugation. The supernatant was decanted and momentarily set aside. The precipitate was dissolved in a minimum of distilled water, reprecipitated with ammonium sulfate, redissolved in distilled water, and the resulting solution dialyzed against running distilled water at 5° C. until free of sulfate. The dialyzed solution was dried by lyophilization. Concentrates from *B. dermatitidis* and *H. capsulatum* were treated similarly, except that $(NH_4)_2SO_4$ was added in the form of a saturated solution to give 90 per cent saturation. In both of these, the addition of solid $(NH_4)_2SO_4$ gave precipitates that had little tendency to flocculate and hence were difficult to centrifuge.

The supernatant from the first $(NH_4)_2SO_4$-precipitation procedure in all cases was diluted 25 to 30-fold with distilled water and concentrated again by the ultrafiltration process already described. When reduced to a convenient volume, the solution was treated with three volumes of 95 per cent alcohol. At this stage the $(NH_4)_2SO_4$ was sufficiently dilute to remain substantially in solution. The precipitate resulting from the addition of alcohol was allowed to settle and then recovered by decantation and centrifugation. The precipitate was dissolved in a minimum of distilled water and reprecipitated, as before, with alcohol. The precipitate was redissolved in distilled water and the resulting solution was dialyzed and lyophilized as described for the $(NH_4)_2SO_4$-precipitated materials.

At no time were we able to obtain a precipitate by the addition of $(NH_4)_2SO_4$ to a concentrated culture filtrate of *C. albicans.* Trichloroacetic acid produced only a slight opalescence. The addition of alcohol, on the other hand, precipitated a material rich in carbohydrate. Fractions were obtained by precipitating with 33 per cent and 33 to 75 per cent alcohol by volume below 0° C., centrifuged in the cold, dissolved in 25 ml. cold water, Seitz filtered, and stored in the freezer. Because only the 33 per cent alcohol fraction gave positive biuret and Millon's tests, a portion of this fraction was treated with four volumes of saturated $(NH_4)_2SO_4$ solution and the precipitated material isolated by centrifugation. The precipitate was dissolved in water and dialyzed against distilled water in the refrigerator until all the sulfate was removed. The supernatant was diluted to 1,000 ml. and subjected to ultrafiltration. When the volume was reduced to 75 ml. an equal quantity of water was added and the mixture reduced by ultrafiltration to 7 milliliters. Three volumes of 95 per cent alcohol were added and the remaining precipitate isolated by centrifugation, dissolved in 24 ml. of water, Seitz filtered, and stored in the freezer.

The yield of dry material from *T. mentagrophytes, T. rubrum, B. dermatitidis,* and *H. capsulatum* was of the order of 0.2 Gm. per liter of original

medium, being approximately equally divided between the $(NH_4)_2SO_4$ and alcohol fractions. *C. albicans* produced about 0.1 Gm. nondialyzable material per liter of original medium.

Analysis of Dried Products: All of the $(NH_4)_2SO_4$-precipitated materials gave positive Millon's and Molisch's tests. The biuret test was positive for materials from *T. mentagrophytes* No. 599, *T. rubrum,* and *H. capsulatum;* in the case of *T. mentagrophytes* No. 640 and *B. dermatitidis* the results of this test were obscured by interfering color reactions.

All of the alcohol-precipitated materials gave positive Molisch's tests and negative biuret and Millon's tests.

The 33 per cent alcohol fraction of the ultrafiltrate of *C. albicans* gave positive Molisch, biuret, and Millon's tests, whereas the 33 to 75 per cent alcohol fraction gave only a positive Molisch's test.

The chemical analysis of all antigenic fractions have been reported elsewhere.[7, 8]

V. *Immunization of Animals.*—A suspension of live spores of *T. mentagrophytes* No. 599, *T. mentagrophytes* No. 640, *T. rubrum, B. dermatitidis,* and *H. capsulatum* was prepared. Ten plates of dextrose agar were inoculated with each of the fungi. The plates inoculated with *T. mentagrophytes* No. 599 and No. 640 and *T. rubrum* were allowed to grow at 30° C. for ten days. The plates inoculated with *B. dermatitidis* and *H. capsulatum* were allowed to grow for four and six weeks, respectively, at 30° Centigrade. A spore suspension in physiologic salt solution was made by scraping the fungous mats from the dextrose agar plates with a nichrome wire needle. Care was taken in making the scrapings to avoid the removal of agar. The material was filtered through sterile cotton to remove mycelial filaments and then the density of the spore suspension was determined by making a spore count. The spore counts were made by employing the technique and apparatus customarily used for the enumeration of red corpuscles. The spore suspensions were then adjusted to the desired value by the addition of sterile physiologic salt solution. Five slants of dextrose agar were inoculated with *C. albicans* and incubated at 30° C. for ten days. Two milliliters of physiologic salt solution were added to each culture tube and by the process of shaking and mixing with a bacteriologic loop a suspension of yeast cells was made. The yeast cell count in the suspension was enumerated as already described.

The scheme of immunization with the live spore suspensions of the fungi differed only in the dosages and in duration of time. Adult albino rabbits of approximately the same size and age were employed and all injections were made intravenously every five to eight days. From two to nine rabbits were immunized with the spores of each fungus and at the end of the periods of immunization, blood was withdrawn for precipitin studies.

The rabbits immunized with *T. mentagrophytes* No. 599 received at first 1 million spores and the dose was gradually increased in multiples of 5 or 10 until finally 100 million spores were given with each injection. The period of immunization was twelve months.

The animals immunized with *T. mentagrophytes* No. 640 and *T. rubrum* were given first 1 million spores and the dose was gradually increased to 10 million spores. The periods of immunization were four months.

The animals immunized with *C. albicans* received 10 million yeast cells for a period of four months.

The animals immunized with *B. dermatitidis* and *H. capsulatum* were started with 10 thousand spores and 50 spores, respectively, and the doses gradually increased to a maximum for both of 10 million spores. The period of immunization in both instances was five months.

VI. *Skin Tests.*—The immunized animals were skin-tested with the $(NH_4)_2SO_4$-precipitated fraction (hereafter referred to as protein fraction) and the alcoholic precipitate of the supernatant (hereafter referred to as carbohydrate fraction) of the *T. mentagrophytes* No. 599 broth concentrate. A 1:10 dilution of each fraction was prepared and employed as skin-testing antigens. The animals were skin-tested from one to three days after they had been bled. The reactions were observed, measured, and recorded thirty minutes, 1, 2, 3, 4, 5, 6, 12, 24, 48 and 96 hours after the skin tests were made.

VII. *Determination of Precipitative Antibody Titers.*—The collodion particle agglutination technique was used for the most part to determine the precipitative titers of rabbit antisera. In some instances the usual method of determining precipitins was employed. The antigenic material was that obtained by precipitating the broth concentrates of the various fungi with $(NH_4)_2SO_4$, which will be hereafter referred to as protein fraction throughout the paper. In regard to the collodion particle agglutination technique, we followed exactly the procedure outlined by Cannon and Marshall.[9]

RESULTS

In Tables I, II, III, IV, V, and VI are recorded the results of serologic tests with varying types of rabbit antisera and the antigens isolated from the concentrated broth filtrates of *T. mentagrophytes* No. 599, *T. mentagrophytes* No. 640, *T. rubrum*, *C. albicans*, *B. dermatitidis*, and *H. capsulatum*. The reactions were highly specific and no cross reactions were observed. The fact that cross reactions between the two species of the Trichophyton genera, *T. mentagrophytes* No. 599 and *T. rubrum*, did not occur was particularly interesting. Furthermore, *T. mentagrophytes* No. 599 "protein" antigen did not react with *T. mentagrophytes* No. 640 antiserum. *T. mentagrophytes* No. 599 is pathogenic while *T. mentagrophytes* No. 640 is not pathogenic for rabbits and guinea pigs. It is to be noted from Table II, however, that precipitins could not be demonstrated in *T. mentagrophytes* No. 640 antiserum with "protein" antigen isolated from the broth centrate of *T. mentagrophytes* No. 640. When precipitin studies were carried out employing the usual technique in place of the collodion particle agglutination method, slight turbidity was noted in all immune antisera, including the normal serum, as well as the *T. mentagrophytes* No. 640 antiserum. Perhaps the fact that the organism is not pathogenic is at least partially responsible for its apparent inability to stimulate the formation of precipitative antibodies. It did occur to us that since the *T. mentagrophytes* No. 599 animals had been

TABLE I. DEMONSTRATION OF PRECIPITATIVE TITERS OF VARIOUS RABBIT ANTISERA EMPLOYING THE COLLODION PARTICLE AGGLUTINATION TECHNIQUE AND THE PROTEIN ANTIGEN DERIVED FROM T. MENTAGROPHYTES NO. 599

TYPES OF RABBIT ANTISERA EMPLOYED	SERIAL DILUTIONS OF ANTISERA							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
T. mentagrophytes No. 599	++	++	++	++	++	++	+	–
T. mentagrophytes No. 640	–	–	–	–	–	–	–	–
T. rubrum	–	–	–	–	–	–	–	–
C. albicans	–	–	–	–	–	–	–	–
B. dermatitidis	–	–	–	–	–	–	–	–
H. capsulatum	–	–	–	–	–	–	–	–
Normal control	–	–	–	–	–	–	–	–

TABLE II. DEMONSTRATION OF PRECIPITATIVE TITERS OF VARIOUS RABBIT ANTISERA EMPLOYING THE COLLODION PARTICLE AGGLUTINATION TECHNIQUE AND THE PROTEIN ANTIGEN DERIVED FROM T. MENTAGROPHYTES NO. 640

TYPES OF RABBIT ANTISERA EMPLOYED	SERIAL DILUTIONS OF ANTISERA							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
T. mentagrophytes No. 599	–	–	–	–	–	–	–	–
T. mentagrophytes No. 640	–	–	–	–	–	–	–	–
T. rubrum	–	–	–	–	–	–	–	–
C. albicans	–	–	–	–	–	–	–	–
B. dermatitidis	–	–	–	–	–	–	–	–
H. capsulatum	–	–	–	–	–	–	–	–
Normal control	–	–	–	–	–	–	–	–

TABLE III. DEMONSTRATION OF PRECIPITATIVE TITERS OF VARIOUS RABBIT ANTISERA, EMPLOYING THE COLLODION PARTICLE AGGLUTINATION TECHNIQUE AND THE PROTEIN ANTIGEN DERIVED FROM T. RUBRUM

TYPES OF RABBIT ANTISERA EMPLOYED	SERIAL DILUTIONS OF ANTISERA							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
T. mentagrophytes No. 599	–	–	–	–	–	–	–	–
T. mentagrophytes No. 640	–	–	–	–	–	–	–	–
T. rubrum	+	+	+	+	–	–	–	–
C. albicans	–	–	–	–	–	–	–	–
B. dermatitidis	–	–	–	–	–	–	–	–
H. capsulatum	–	–	–	–	–	–	–	–
Normal control	–	–	–	–	–	–	–	–

TABLE IV. DEMONSTRATION OF PRECIPITATIVE TITERS OF VARIOUS RABBIT ANTISERA EMPLOYING AS AN ANTIGEN THE 1:100 DILUTION OF AN ALCOHOL PRECIPITATE FROM C. ALBICANS BROTH CONCENTRATE (ORDINARY PRECIPITIN TECHNIQUE USED)*

TYPES OF RABBIT ANTISERA EMPLOYED	SERIAL DILUTIONS OF ANTISERA							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
T. mentagrophytes No. 599	–	–	–	–	–	–	–	–
T. mentagrophytes No. 640	–	–	–	–	–	–	–	–
T. rubrum	–	–	–	–	–	–	–	–
C. albicans	++++	++++	+++	++	+	–	–	–
B. dermatitidis	–	–	–	–	–	–	–	–
H. capsulatum	–	–	–	–	–	–	–	–
Normal control	–	–	–	–	–	–	–	–

*Tubes incubated at 96° F. for seven hours and read after standing overnight in refrigerator.

TABLE V. DEMONSTRATION OF PRECIPITATIVE TITERS OF VARIOUS RABBIT ANTISERA EMPLOYING AS AN ANTIGEN THE 1:10 DILUTION OF PROTEIN ISOLATED FROM B. DERMATITIDIS BROTH CONCENTRATE (ORDINARY PRECIPITIN TECHNIQUE USED)*

TYPES OF RABBIT ANTISERA EMPLOYED	SERIAL DILUTIONS OF ANTISERA							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
T. mentagrophytes No. 599	–	–	–	–	–	–	–	–
T. mentagrophytes No. 640	–	–	–	–	–	–	–	–
T. rubrum	–	–	–	–	–	–	–	–
C. albicans	–	–	–	–	–	–	–	–
B. dermatitidis	++	+	–	–	–	–	–	–
H. capsulatum	–	–	–	–	–	–	–	–
Normal control	–	–	–	–	–	–	–	–

*Tubes incubated at 94° F. for eight hours and read after standing overnight in refrigerator.

TABLE VI. DEMONSTRATION OF PRECIPITATIVE TITERS OF VARIOUS RABBIT ANTISERA, EMPLOYING THE COLLODION PARTICLE AGGLUTINATION TECHNIQUE AND THE HISTOPLASMA CAPSULATUM BROTH CONCENTRATE

TYPES OF RABBIT ANTISERA EMPLOYED	SERIAL DILUTIONS OF ANTISERA							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
T. mentagrophytes No. 599	–	–	–	–	–	–	–	–
T. mentagrophytes No. 640	–	–	–	–	–	–	–	–
T. rubrum	–	–	–	–	–	–	–	–
C. albicans	–	–	–	–	–	–	–	–
B. dermatitidis	–	–	–	–	–	–	–	–
H. capsulatum	–	–	–	–	–	–	–	–
Normal control	–	–	–	–	–	–	–	–

TABLE VII. DEMONSTRATION OF THE DEVELOPMENT TIME OF PRECIPITATIVE ANTIBODIES AFTER REPEATED INTRAVENOUS INJECTIONS OF T. MENTAGROPHYTES NO. 599 SPORES. T. MENTAGROPHYTES NO. 599 PROTEIN ANTIGEN AND ORDINARY PRECIPITIN TECHNIQUE EMPLOYED

RABBIT NO.	NUMBER OF SPORES INJECTED WITH DATE OF INJECTION	DATE OF BLEEDING AND DETERMINATION OF PRECIPITINS	SERIAL DILUTIONS OF ANTISERA				
			SERUM	1:2	1:4	1:8	1:16
33	100,000 1/16/47	1/14/47	–	–	–	–	–
40	100,000 1/16/47	1/14/47	–	–	–	–	–
33	1,000,000 1/20/47	1/23/47	–	–	–	–	–
40	1,000,000 1/20/47	1/23/47	–	–	–	–	–
33	10,000,000 1/25/47	1/30/47	+	–	–	–	–
40	10,000,000 1/25/47	1/30/47	+	±	–	–	–
33	6,000,000 1/31/47	2/ 6/47	+	±	–	–	–
40	6,000,000 1/31/47	2/ 6/47	+	±	–	–	–
33	23,000,000 2/7/47	2/20/47	+	±	–	–	–
40	23,000,000 2/7/47	2/20/47	+	±	–	–	–
33	20,000,000 2/13/47	3/ 6/47	++	+	–	–	–
40	20,000,000 2/13/47	3/ 6/47	++	+	±	–	–

TABLE VIII. DEMONSTRATING DIAMETER OF SKIN REACTIONS, IN MILLIMETERS, AT VARIOUS INTERVALS IN IMMUNIZED RABBITS AFTER THE INTRADERMAL INJECTION OF 0.1 ML. OF I: 1:10 DILUTION OF T. MENTAGROPHYTES No. 599 PROTEIN, OF II: 1:10 DILUTION OF T. MENTAGROPHYTES No. 599 CARBOHYDRATE

TYPE OF IMMUNIZED RABBIT	TIME INTERVALS OF READING SKIN TESTS AFTER INJECTION OF ANTIGENS AND TYPES OF ANTIGENS EMPLOYED																					
	½ HOUR		1 HOUR		2 HOURS		3 HOURS		4 HOURS		5 HOURS		6 HOURS		12 HOURS		24 HOURS		48 HOURS		96 HOURS	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
T. mentagrophytes No. 599	15		15		18		20		25		20		25		20		20		18		10	
		15		15		13		17		17		17		15		15		12		12		8
T. mentagrophytes No. 640	18		18		20		25		30		30		25		25		15		10		0	
		15		15		13		13		15		15		12		15		12		10		5
T. rubrum	15		15		20		20		25		25		22		20		25		20		12	
		15		12		10		12		10		10		10		12		10		10		0
C. albicans	15		14		20		20		25		20		20		15		12		10		8	
		15		12		8		10		10		12		10		8		7		5		5
B. dermatitidis	18		18		18		25		20		25		18		20		20		10		0	
		15		10		12		10		12		12		8		10		8		5		0
H. capsulatum	15		16		17		20		20		20		15		20		20		10		0	
		15		14		10		10		13		10		7		8		5		5		0
Normal control	13		15		15		20		20		20		20		20		15		15		0	
		15		17		15		18		18		18		15		12		6		5		0

immunized over a period of twelve months and the *T. mentagrophytes* No. 640 animals had been immunized for a period of only four months we had not allowed sufficient time for the stimulation and production of precipitin antibodies in the *T. mentagrophytes* No. 640 animals. Table VII presents data on the development time of precipitins in two rabbits immunized with *T. mentagrophytes* No. 599. Precipitins appeared in both animals two weeks after their first intravenous injection of spores. Both animals had received three injections during this two-week period. The knowledge of the specificity of the precipitin reactions, demonstrated by *T. mentagrophytes* No. 599 and *T. rubrum,* may lend itself as a useful adjunct to morphologic criteria for the classification of species in the genus Trichophyton. It is also possible that such methods will prove useful in the classification of various strains of the same species in regard to their pathogenicity, in much the same manner as bacteria have been grouped by serologic methods.

We failed to demonstrate precipitative antibodies in the sera of animals immunized with *H. capsulatum* and this detracts from the fact that *B. dermatitidis* protein gave precipitin reactions against *B. dermatitidis* antiserum but not against *H. capsulatum* antiserum. It is to be remembered that in skin testing with histoplasmin and blastomycin, cross reactions are unfortunately frequent.

In Table VIII are recorded the results of skin testing the variously immunized rabbits with both protein and carbohydrate fractions isolated from *T. mentagrophytes* No. 599 broth concentrate. There is no appreciable difference in the size or character of the skin reactions in any of the animals to either the protein or carbohydrate fractions until a period of forty-eight hours has elapsed. When the tests were read after forty-eight hours the animals immunized to *T. mentagrophytes* No. 599 and *T. rubrum* reacted similarly but different from the other animals whose reactions closely paralleled the skin reactions in the normal control. After ninety-six hours the skin reactions in the *T. mentagrophytes* No. 599 and *T. rubrum* animals were still marked while the skin reactions had completely subsided in all other animals except for a mild reaction to the carbohydrate fraction in the *T. mentagrophytes* No. 640 rabbit and positive reactions to both fractions in the *C. albicans* rabbit. From purely the results of the skin tests in these few animals it might be suggested that *T. mentagrophytes* No. 599 antigen gives cross reactions in animals immunized with *T. mentagrophytes* No. 640, *T. rubrum,* and *C. albicans.* However, it is hazardous to rely on skin testing to determine the specificity of an antigen and this is clearly demonstrated by reviewing our serologic studies which precisely revealed the specificity of the various antigenic fractions.

We were never able to obtain a precipitate by the addition of $(NH_4)_2SO_4$ to a concentrated culture filtrate of *C. albicans.* A chemical analysis[7, 8] of the dialyzed culture-filtrate concentrate revealed 2.9 per cent nitrogen, which was comparable in nitrogen content to the carbohydrate fractions isolated from the other organisms. Also the percentage of reducing substance, calculated as glucose, was comparable to the per cent of reducing substance in the alcohol-precipitated fractions of the other fungi. It seemed likely, therefore, that a goodly

portion of the extracellular substance was made up of carbohydrate. A fraction of the broth concentrate was obtained by precipitating with 33 per cent alcohol and another fraction was obtained by precipitating the supernatant with 75 per cent alcohol. The 33 per cent alcohol fraction gave positive biuret and Millon's tests as well as a positive Molisch's test. The 33 to 75 per cent alcohol fraction gave only a positive Molisch's test. From the 33 per cent alcohol fraction was obtained a precipitate on the addition of $(NH_4)_2SO_4$ and this was called "protein." The supernatant was treated with 95 per cent alcohol and a precipitate was isolated which we called "protein-free" fraction. The sensitizing abilities of the broth concentrate, the protein, the protein-free, the 33 to 75 per cent alcohol fractions, and Oidiomycin were compared by making intradermal injections of these antigens into guinea pigs at four-day intervals. On each occasion of skin testing the resulting reactions were observed 1, 2, 3, 4, 5, 6, 12, 24, 48, and 96 hours after testing. The results of this experiment are recorded in Table IX.

Anaphylactic hypersensitivity was demonstrated in the animal that had received an intradermal injection of the 33 to 75 per cent alcohol fraction four

TABLE IX. DEMONSTRATION OF SENSITIZING POTENTIALITIES OF VARIOUS ANTIGENS DERIVED FROM C. ALBICANS BROTH FILTRATES

DATE OF INJECTION (INTRADERMAL) OF ANTIGEN	GUINEA PIG NO.	ANTIGEN	TIME OF READING (IN HOURS) AND SIZE (IN MM.) OF SKIN REACTIONS									
			1	2	3	4	5	6	12	24	48	96
First injection: Aug. 29, 1947	31	1*	12	15	15	15	13	13	10	7	4	0
	32	2	20	20	20	22	22	25	27	23	25	0
	33	3	10	10	8	9	10	13	13	17	17	0
	34	4	10	10	8	9	10	12	14	13	13	0
	35	5	10	10	11	12	12	15	15	17	18	0
Second injection: Sept 2, 1947	31	1	13	14	15	13	13	14	15	15	12	0
	32	2	18	25	27	30	30	30	28	30	27	0
	33	3	17	15	12	13	15	17	21	23	20	0
	34	4	14	12	10	10	11	12	14	14	13	0
	35	5	19	25	30	30	33	35	35	35	30	0
Third injection: Sept. 6, 1947	31	1	12	12	12	12	12	13	15	17	12	0
	32	2	20	23	27	28	30	30	30	30	15	0
	33	3	18	19	20	21	22	22	25	25	17	0
	34	4	12	11	11	11	12	13	15	14	13	0
	35	5	19	23	27	30	30	32	35	35	15	0
Fourth injection: Sept. 10, 1947	31	1	13	14	16	15	17	15	18	22	13	0
	32	2	19	22	25	27	30	30	33	35	10	0
	33	3	18	20	22	21	22	22	24	25	7	0
	34	4	13	13	12	12	13	14	17	17	12	0
	35	5	19	23	28	30	32	32	35	35	15	0

*1 = 1:10 Oidiomycin.
2 = 1:25 Broth concentrate.
3 = 1:10 Protein from 33 per cent alcohol fraction.
4 = 1:6 Protein-free from 33 per cent alcohol fraction.
5 = 1:5 Alcohol fraction, 33 to 75 per cent.

days after the first injection had been made. This state of hypersensitivity was manifested by the development of edema at the side of the second injection within one to two hours following its administration. Repeated injections of the protein fraction and the broth concentrate were required to produce anaphylactic hypersensitivity and even then the degree of hypersensitivity, as determined by the degree of edema occurring at the site of injection, did not compare with that elicited by the 33 to 75 per cent alcohol fraction. Oidiomycin was a poor sensitizer and the protein-free fraction did not sensitize at all.

TABLE X. COMPARISON OF PRECIPITATIVE TITERS OF GUINEA PIG ANTISERA PREPARED BY SENSITIZING ANIMALS WITH OIDIOMYCIN AND A 33 TO 75 PER CENT ALCOHOL FRACTION FROM A C. ALBICANS CONCENTRATED BROTH FILTRATE

ANTIGENS	GUINEA PIG ANTISERA	SERIAL DILUTIONS OF ANTISERA							
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Oidiomycin, 1:20	Oidiomycin-sensitized	++	+	−	−	−	−	−	−
33 to 75% alcohol fraction *C. albicans*, 1:50	Oidiomycin-sensitized	++	+	±	−	−	−	−	−
C. albicans protein, 1:10	Oidiomycin-sensitized	++	+	±	−	−	−	−	−
Oidiomycin, 1:20	33 to 75% alcohol fraction *C. albicans*-sensitized	+	±	−	−	−	−	−	−
33 to 75% alcohol fraction *C. albicans*, 1:50	33 to 75% alcohol fraction *C. albicans*-sensitized	+++	++	+	+	−	−	−	−
C. albicans protein, 1:10	33 to 75% alcohol fraction *C. albicans*-sensitized	+++	++	+	+	−	−	−	−
Oidiomycin, 1:20	Normal control	−	−	−	−	−	−	−	−
33 to 75% alcohol fraction *C. albicans*, 1:50	Normal control	−	−	−	−	−	−	−	−

Immediately after each guinea pig had received the fourth injection of antigen, they were bled and precipitin studies were carried out. Oidiomycin-sensitized guinea pig serum was tested against Oidiomycin, protein fraction, and the 33 to 75 per cent alcohol fraction; and similarly 33 to 75 per cent alcohol fraction-sensitized guinea pig serum was tested with the 33 to 75 per cent alcohol fraction, protein fraction, and Oidiomycin. The results are tabulated in Table X. It is demonstrated that the highest precipitative antibody titer was obtained in the guinea pig sensitized with the 33 to 75 per cent alcohol fraction. Furthermore, even with the Oidiomycin-sensitized guinea pig serum, the 33 to 75 per cent alcohol fraction and the protein fraction were more sensitive antigens for the demonstration of precipitative antibodies than Oidiomycin. It would seem, therefore, that the 33 to 75 per cent alcohol fraction, which is mostly carbohydrate, contains a high concentration of specific antigenic substance from the broth concentrate of *C. albicans*.

SUMMARY

1. Synthetic media providing both rapid and luxurious growths for *T. mentagrophytes, T. rubrum, C. albicans, B. dermatitidis,* and *H. capsulatum* have been developed.

2. Protein and carbohydrate fractions have been isolated from each of the concentrated broth filtrates of *T. mentagrophytes* No. 599 and No. 640, *T. rubrum, C. albicans, B. dermatitidis,* and *H. capsulatum.*

3. Precipitin reactions, employing either the protein fraction or the broth concentrate from the various fungi and rabbit antisera prepared by inoculating intravenously with live spore suspensions of the fungi, were specific. No cross reactions occurred.

4. Precipitative antibodies were demonstrated in rabbits immunized with *T. mentagrophytes* No. 599 after three injections of live spores given during a period of two weeks.

5. Rabbits immunized with the various fungi were skin tested with the carbohydrate and protein fractions obtained from the broth concentrate of *T. mentagrophytes* No. 599. There were cross reactions in animals immunized with *T. mentagrophytes* No. 599 and No. 640, *T. rubrum,* and *C. albicans.*

6. A 33 to 75 per cent alcohol fraction of *C. albicans* broth concentrate, giving negative tests for protein and a strong Molisch test, sensitized guinea pigs rapidly.

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STUDIES OF CAPILLARY PERMEABILITY IN ALLERGY WITH THE DERMOFLUOROMETER*†

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PART of the mechanism of the reaction in atopic diseases, such as bronchial asthma, hay fever, and atopic dermatitis, is accepted to be due to the action of a histamine-like substance liberated as a result of the antigen-antibody union in the shock tissue. This histamine-like substance acts on the capillaries producing increased capillary dilatation and permeability. In order to understand this mechanism better, it becomes desirable to obtain additional information about the nature and degree of capillary dilatation and permeability which occurs under various conditions. This paper concerns itself with a study of capillary function in allergic and nonallergic patients, using for this purpose the dermofluorometer following the intravenous administration of fluorescein.

ANATOMY AND PHYSIOLOGY OF CAPILLARIES

As a preliminary we shall present a brief review of the anatomy and physiology of the capillaries. Anatomically, capillaries are long thin tubules composed of a single layer of flat endothelial cells held together by an intercellular cement substance. The inner surface of this endothelial wall is lined by an adsorbed layer of serum protein, while the outer surface is covered by a fine sheath.[1, 2] Zweifach and Chambers[3, 4] have studied the mesenteric capillary bed in some detail. They describe two types of capillaries, one being a thoroughfare vessel which is the direct extension from the terminal arterioles and which unites with similar vessels to form venules. A thin layer of muscle cells extends beyond the terminal arteriole to the proximal portion of the thoroughfare vessel, which is called the "metarteriole." The other type, the "true capillary," is a branch of the metarteriole or thoroughfare vessel and empties into the distal portion of the thoroughfare vessel or into a venule. (Fig. 1.) No muscle layer envelops the true capillary, but at its origin the endothelium is surrounded by muscle cells which form the "precapillary sphincter." The capillaries, the functional portion of the vascular system, allow the filtration, absorption, and diffusion of gases, water, electrolytes, and certain other substances between the blood and tissue fluid.

Capillaries are independently able to contract and dilate, probably depending on chemical, nervous, and perhaps other stimuli, the most notable of which occurs when the tissue passes from rest into an active state and the metabolic requirements are increased. In such a state of activity a far greater number of true capillaries can be seen due to the relaxation of the precapillary sphincters, which at rest undergo intermittent contraction and dilatation producing

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an intermittent flow in the true capillary. Metarterioles also undergo intermittent vasomotion, but the contraction is not complete so that the thoroughfare channels always remain open. The endothelial cell itself is capable to some extent of swelling but not enough to occlude an active circulation.[5] Epinephrine causes a contraction of the precapillary sphincters and the metarterioles, while histamine dilates these muscular elements. Krogh[6] postulated that capillary tone might be maintained by a hormone probably from the pituitary. Lewis[7] described the response of the skin to mechanical stimulation as the "triple response," that is, the red line, the flare, and the wheal. Since this reaction is the same as the response of the skin to the intradermal injection of histamine, he suggested that it is due to liberation of histamine or a histamine-like substance. This substance is also considered responsible for manifestations of anaphylaxis and atopy.

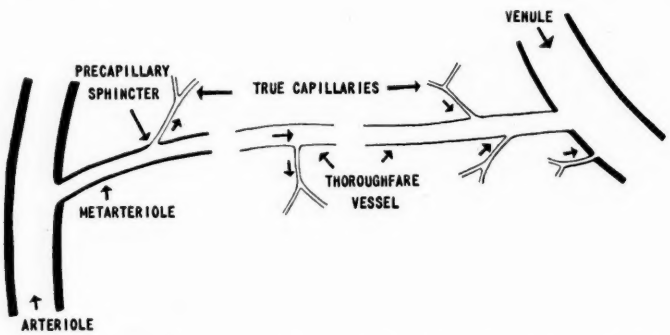

Fig 1.—Scheme of capillary circulation modified after Chambers and Zweifach.

The color of the skin, apart from pigmentation, depends not upon the blood in the capillary loops so much as the amount of blood in the subcapillary venous plexuses. The temperature of the skin depends on the rate of blood flow. Moderate degrees of heat dilate capillaries and increase speed of flow, while cold contracts the arterioles, slows blood flow, and causes the capillaries to become empty. Excesses of either heat or cold injure the capillary walls. The passage of fluids and other substances such as electrolytes through the capillary wall demonstrates capillary permeability and is to be differentiated from capillary fragility which refers to the passage of red cells.[8] These two phenomena do not necessarily go together, for at times fluid alone passes through a capillary as occurs in formation of a wheal, and at other times red blood cells pass through to produce petechiae or purpura. The degree of capillary permeability is measured by the amount of a substance which passes through the capillary wall in a certain time and this depends in part upon the area and the thickness of the capillary wall and the difference in pressure inside and outside of the capillary.[9] The direction of flow of water and solutes, that is, filtration or absorption, depends on the balance between the hydrostatic and colloid osmotic pressure. Diffusion of molecules may occur in either direction with either absorption or filtration and involves the movement of a substance from a region of high concentration to a region of low concentration.

Between the endothelial cells are pores of various sizes through which molecules pass, the sizes determining which molecules will pass. According to Chambers and Zweifach[10] the character of the endothelial cement also appears to play a role in what passes through these pores. Increased acidity and deficiency of calcium alters the stability of this cement and increases capillary permeability. There is a gradient of permeability at least to dyes, increasing from the arterial to the venous end of the capillary, which depends neither on pressure nor structural differences in the wall, but possibly on something in the blood at the venous end.[2] Severe anoxia, certain chemicals, and perhaps shock are other factors which are known to increase capillary permeability.

FLUORESCEIN IN THE STUDY OF CAPILLARY FUNCTION

No way exists to obtain or measure the true capillary filtrate, hence no direct measurement exists for the determination of the permeability of the capillary wall. Present estimations of capillary permeability are only by indirect methods. Although others have used dyes as indicators of permeability, Landis[8] considers their use reliable only under best controlled conditions.

In 1943, Lange and Krewer[11] devised the dermofluorometer, an instrument which can be used for objective determination of circulation time and capillary permeability. This instrument, which consists of an ultraviolet light source and a photoelectric cell, measures the intensity of fluorescence at a skin site and this is expressed as "skin units" based upon the method of standardizing the instrument. Following the intravenous administration of fluorescein, which diffuses immediately into the interstitial spaces, fluorescence curves can be obtained which fall in a certain range in normal persons. The first part of the curve depends upon the amount of blood reaching the area per unit time, as well as upon the intracapillary pressure and capillary permeability and the concentration of fluorescein in the blood. The latter part of the curve may depend upon renal excretion of the dye. Lange found low curves in arteriosclerosis[12] where the blood flow is reduced, and high curves in myxedema[13] where factors other than increased capillary permeability were ruled out as causes. He also found an increased fluorescence in areas of inflammation and histamine whealing, which was due to increased permeability. In a cooled area, though hyperemic, he noted decreased fluorescence and noted similar concentrations of fluorescein in the capillaries from the cooled and noncooled sites. He concluded the body had decreased permeability as a defense mechanism against freezing. However, other workers have recently presented evidence, obtained by other methods, that capillary permeability was increased by cold.[8]

The following observations deal with measurements of capillary permeability in allergic and nonallergic patients as determined by means of the dermofluorometer following intravenous injection of fluorescein, after the method described by Lange.[13]

TECHNIQUE

Experiments were performed in a quiet, semidarkened room with the temperature varying on different days from 23° to 26° C., but generally constant throughout each test. The subjects were kept in a recumbent position and rested

for ten to twenty minutes prior to beginning the experiment. Each subject had preliminary readings taken with the dermofluorometer over the site of skin to be observed which in these experiments was a shaved area on the anterior surface of the lower third of the thigh, an area chosen because of its large flat surface and accessibility. Intravenous injections were made in the antecubital vein using 1.3 c.c. per 10 kg. of body weight of a 5 per cent solution of fluorescein containing 5 per cent sodium bicarbonate.* Dermofluorometer readings were then taken at frequent intervals for one hour and curves were plotted for each subject, the preliminary reading having been subtracted from the reading taken after injection of the dye. All tests were performed by the same observer using the same dermofluorometer.

RESULTS

A. *Natural Skin Fluorescence.*—All persons have a very small degree of natural skin fluorescence. This is indicated by the preliminary or "background" reading obtained on the skin using the dermofluorometer prior to injection of fluorescein. As a matter of interest we reviewed these background readings in ninety-three of the subjects we tested. These readings varied from 5 to 55 skin units and were to a small degree related to skin coloring. Negroes had very low readings. Our subjects, all white adult males, were roughly divided into three groups: dark, medium, and light. Their readings averaged 15.4, 21.1, and 24.2, respectively. When they were separated further into allergic and nonallergic groups, it was noted that the allergic patients had readings whose average was somewhat less than that of the nonallergic patients, and those allergic patients having urticaria had readings whose average was even lower (Table I). Readings tended to be lower in younger patients. They varied within narrow limits from leg to leg in one patient, and from day to day in the same patient. Injection of 0.1 c.c. of 1:10,000 dilution of histamine produced a drop of several skin

TABLE I. NATURAL SKIN FLUORESCENCE*

	NUMBER OF PATIENTS	DARK (SKIN UNITS)	MEDIUM (SKIN UNITS)	LIGHT (SKIN UNITS)
All patients	93	15.4	21.1	24.2
Nonallergic patients	47	17.8	21.5	24.6
Allergic patients	46	12.5	20.8	23.6
Urticaria patients	14	11.5	14.7	23.2

*Average readings are recorded.

units (averaging 4) within five to ten minutes over the resultant wheal site. Injection of ragweed extract or physiologic saline solution produced even less of a drop in the reading. Application of heat or cold, which was accompanied by erythema, produced a decrease in the background reading, averaging 8 skin units for heat and 2 for cold. Control sites on the opposite thigh were unaffected by either the injections or applications. Pyribenzamine in small doses had no effect on the background readings. These readings are reported without any attempt at interpretation.

B. *Fluorescence After Injection of Fluorescein.*—Using the technique already described, fluorescence curves were plotted for sixteen nonallergic subjects.

*Fluorescite manufactured by C. F. Kirk Co., New York, N. Y.

The range of values and average curve are shown in Fig. 2. These results are similar to those of Lange.[13] The maximum fluorescence was reached from ten to thirty minutes, usually about twenty minutes after injection of fluorescein. Similarly, curves were plotted for eighteen patients with various allergic diseases. Fig. 3 shows the average curve and the limits in these patients. These results are but a little below those of the nonallergic group. Curves drawn for four patients with urticaria included in the allergic group, were similar to those of this group (Fig. 4).

C. *Hyperfluorescence of Histamine Wheals.*—In nine nonallergic subjects, histamine wheals were produced by the intradermal injection of 0.1 c.c. of 1:10,000 dilution of histamine in the test site on the thigh ten minutes prior to the intravenous injection of fluorescein. The range of values and average curve are shown in Fig. 5. When compared with control sites there was marked fluorescence in every instance. The maximum fluorescence was reached several minutes earlier over the wheal sites and then diminished a little more quickly than over normal skin. Similar results were obtained in thirteen allergic patients, including two patients with urticaria (Fig. 6).

D. *Fluorescence of Wheal Sites in Relation to Origin and Size.*—A series of patients was injected intradermally with several materials that produce wheals and ten minutes following this injection, when the wheals were at their maximum size, fluorescein was given intravenously and the fluorescence noted as in the previous experiments.

1. Two nonallergic and two allergic patients were given intradermal injections of 0.05 c.c. and 0.1 c.c. of 1:10,000 dilution of histamine, the former producing wheals averaging 15 × 13 mm. in size, and the latter producing wheals averaging 22 × 17 millimeters. In every case there was hyperfluorescence over both wheal sites in contrast to a normal site, the larger wheal having more fluorescence than the smaller. No differences were noted between allergic or nonallergic patients.

2. In four allergic (ragweed-sensitive) patients and one nonallergic patient, wheals were made using 0.1 c.c. of 1:10,000 dilution of histamine and 0.1 c.c. of 0.001 mg. N ragweed extract. The histamine wheals averaged 23 × 18 mm. in size. In the nonallergic patient, ragweed induced no whealing but a small elevation 9 × 9 mm. was present. All four allergic patients developed ragweed wheals averaging 18 × 13 millimeters. In only one of these patients was the ragweed wheal larger than the histamine wheal. In all cases the larger wheal was more fluorescent, both wheals fluorescing more than the normal skin. In the nonallergic patient, the ragweed-injected site had a slight increase in fluorescence over the normal.

3. In two nonallergic patients, intradermal injections of 0.1 c.c. of 1:10,000 dilution of histamine and 0.1 c.c. physiologic saline were made. Histamine wheals averaging 23 × 17 mm. in size and elevations from the saline averaging 10 × 9 mm. resulted. The histamine wheals were greatly hyperfluorescent, but the saline areas had only a small increase in fluorescence when compared with normal areas. Two allergic patients were similarly injected with physiologic saline, with corresponding results.

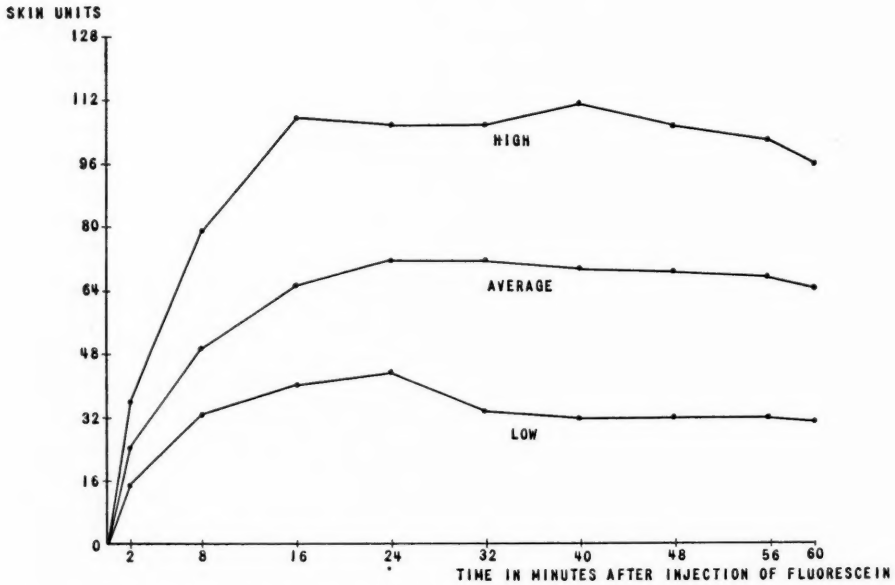

Fig. 2.—Fluorescence after injection of fluorescein. Normal curves in sixteen subjects.

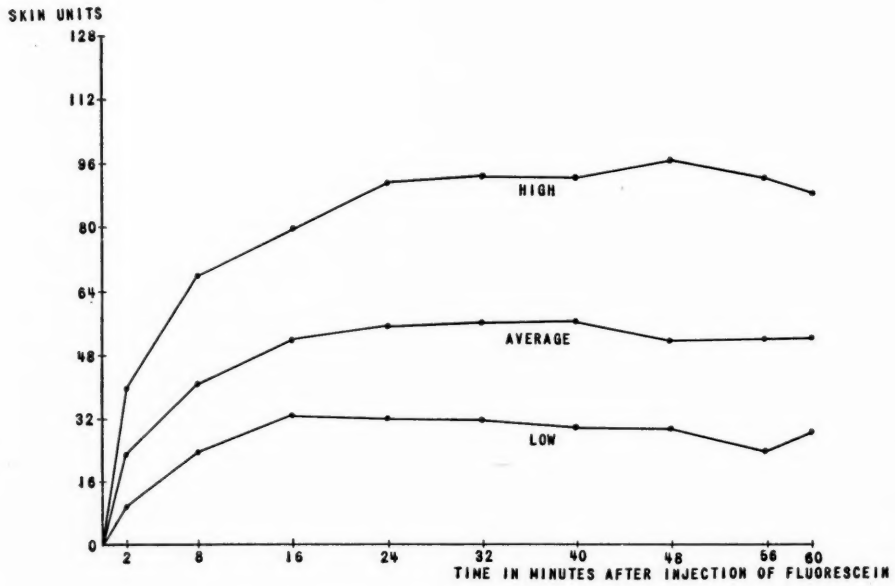

Fig. 3.—Fluorescence in eighteen allergic patients.

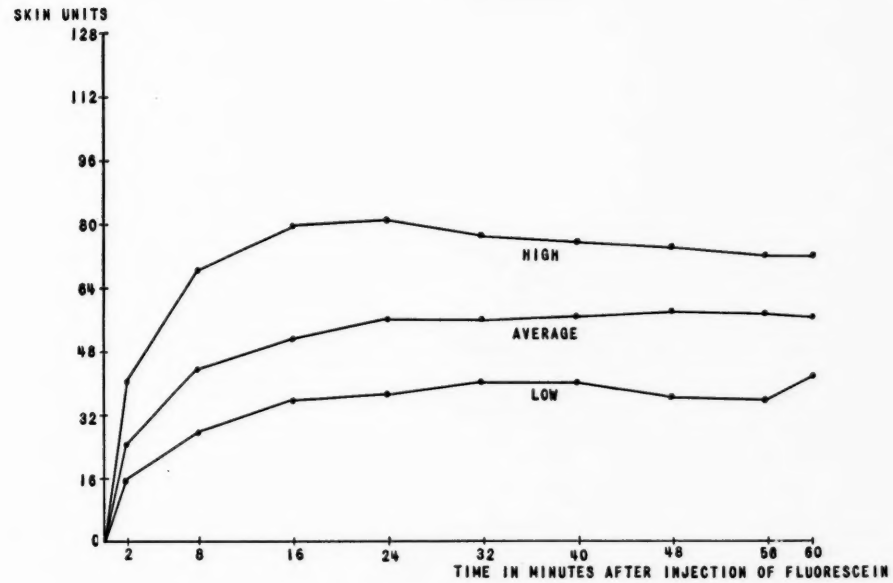

Fig. 4.—Fluorescence in four patients with urticaria.

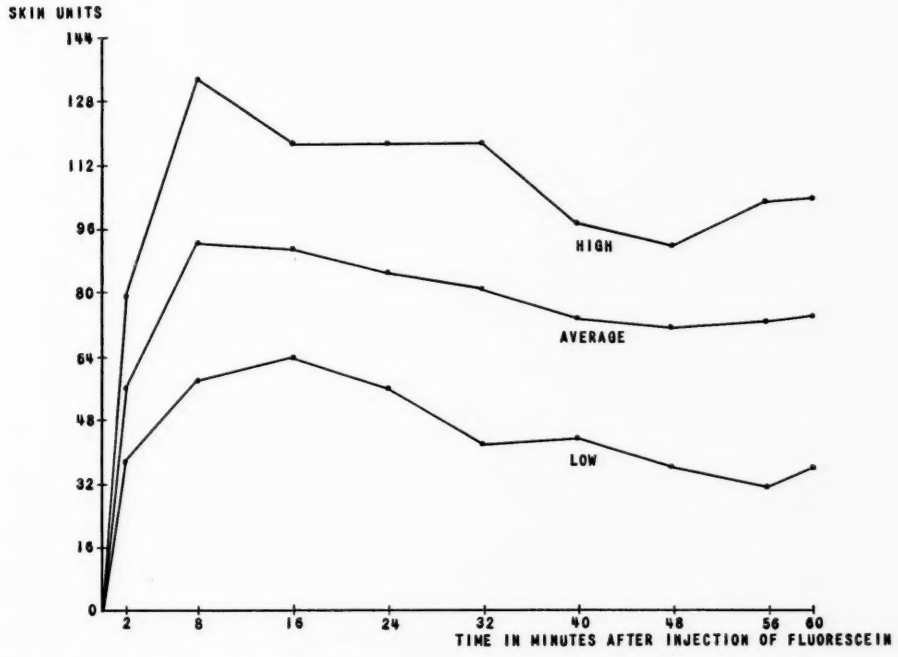

Fig. 5.—Hyperfluorescence of histamine wheals. Nine nonallergic subjects administered 0.1 c.c. histamine.

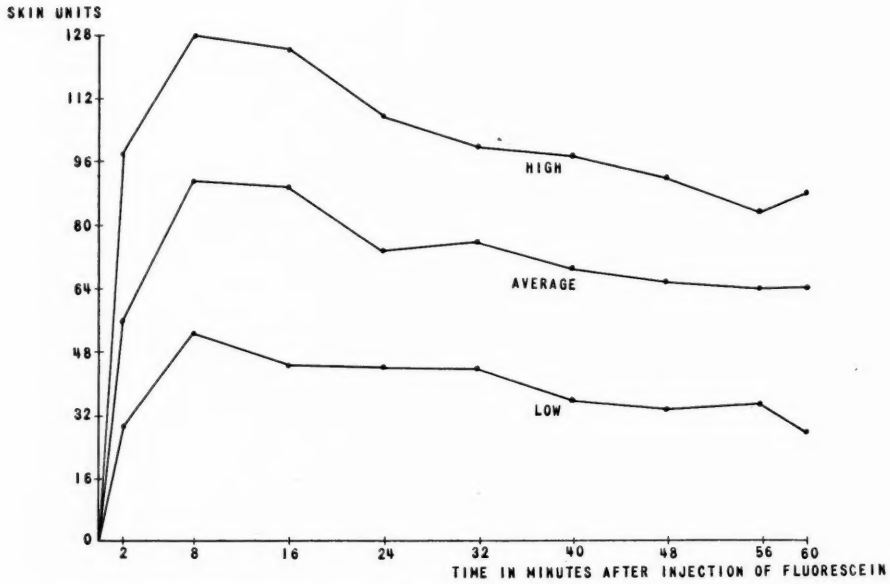

Fig. 6.—Hyperfluorescence over histamine wheals in thirteen allergic patients.

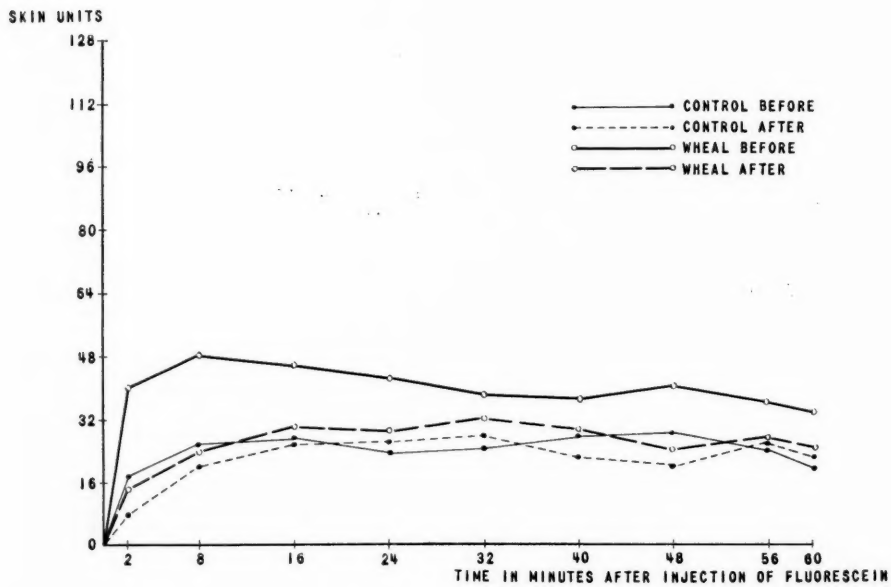

Fig. 7.—Effect of epinephrine.

E. *Fluorescence of Urticarial Wheals.*—Fluorescence curves were obtained in four patients with chronic urticaria; the lesions were compared with adjacent uninvolved areas. In every case there was hyperfluorescence over the urticarial wheal. In one allergic patient with an associated dermographia, an area of dermographic whealing was made by scratching the abdomen. This area had greater fluorescence than an adjacent uninvolved area after fluorescein was injected.

F. *Effect of Epinephrine on Fluorescence.*—One allergic and one nonallergic patient were given 0.7 c.c. and 0.5 c.c., respectively of 1:1,000 dilution of epinephrine HCl subcutaneously seven minutes before intradermal injection of 0.05 and 0.1 c.c. of 1:10,000 dilution of histamine. Ten minutes after injecting the histamine, fluorescein was given. In the allergic patient, the wheals produced were much smaller than those produced on another occasion without epinephrine, and not much difference in area was noted between them. The fluorescence curves were not significantly different over either wheal site or over an adjacent normal site. In the similar test done previously without epinephrine, this patient showed definite hyperfluorescence over the wheal sites, greater in the larger wheal. (Fig. 7.) The nonallergic patient developed wheals of the expected size and these fluoresced more than the normal skin only in the first ten minutes after injection of the dye. Subsequently, all the curves were almost superimposed on one another.

G. *Effect of Pyribenzamine on Fluorescence.*—A group of four allergic and four nonallergic patients were given 200 mg. of Pyribenzamine ten minutes prior

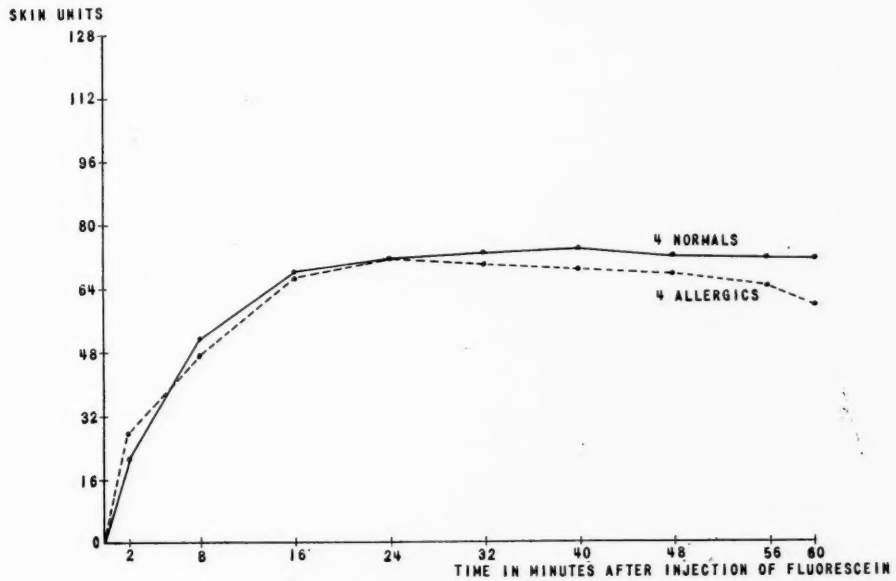

Fig. 8.—Effect of Pyribenzamine on fluorescence. Administration of 200 mg. ten minutes before dye.

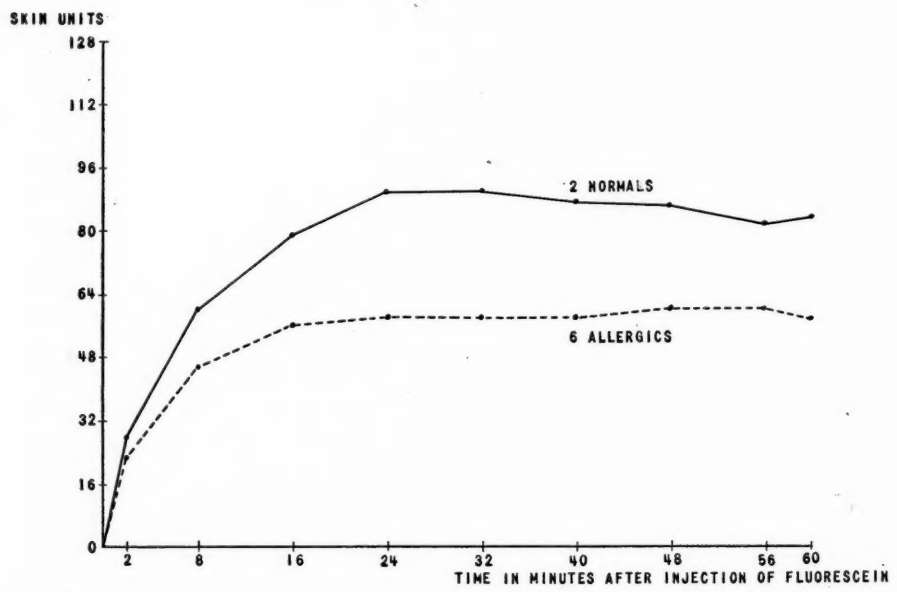

Fig. 9.—Effect of Pyribenzamine on fluorescence. Administration of 200 mg. thirty minutes before dye.

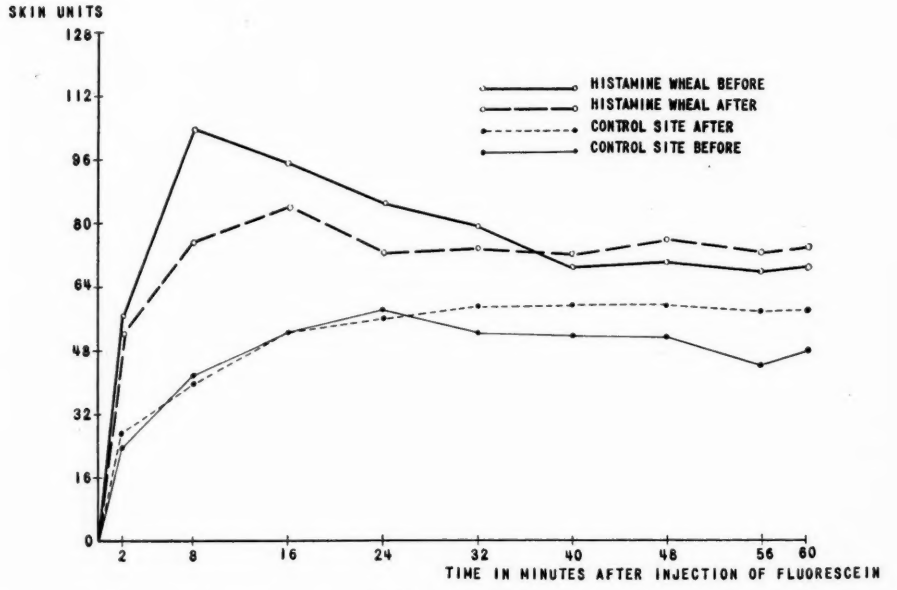

Fig. 10.—Effect of Pyribenzamine on fluorescence. Administration of 600 mg. daily for three days.

to the intravenous administration of fluorescein. Fluorescence curves were not affected. (Fig. 8.) Likewise, when six allergic and two nonallergic subjects were given 200 mg. of Pyribenzamine thirty minutes before the dye injection, no changes resulted (Fig. 9). Even when one normal and three allergic patients were given 600 mg. of Pyribenzamine daily for three days prior to the test no change resulted in the fluorescence curves for normal skin. However, there was a decrease in fluorescence over histamine wheal sites. (Fig. 10.)

DISCUSSION

The fluorescence curves in the nonallergic subjects studied here were similar to those obtained by Lange. No significant difference from normal was observed in patients with various allergic diseases, including urticaria. This would indicate the absence of any increased capillary permeability per se in allergic individuals.

Histamine wheals were hyperfluorescent to the same degree in nonallergic and allergic patients. The direct action of histamine is on the precapillary sphincters producing relaxation which results in capillary dilatation. Distention and stretching of the capillary wall has a tendency to enlarge the pores, decrease the thickness of the capillary wall, and thus increase permeability. Dilatation from any cause should increase permeability. Consequently, histamine wheals secondarily increase permeability, allowing transudation of fluid from the capillary into the interstitial spaces just below the epidermis. Following intravenous administration, fluorescein readily diffuses through this already hyperpermeable membrane into the wheal fluid. This fluid is of far greater volume than that underneath normal skin where the diffusion occurs to lesser degree with lesser fluorescence. Furthermore, increased intracapillary pressure has been demonstrated in the flare of the histamine wheal. This increased pressure accompanies capillary dilatation and adds to the accumulation of wheal fluid. As a rule, allergic and nonallergic patients develop wheals of comparable size from a given intradermal dose of histamine. This explains the same degree of hyperfluorescence in both groups. It suggests that the capillaries of allergic individuals react to histamine in the same manner as normal persons.

Hyperfluorescence occurred over wheals regardless of mode of production, and the degree of hyperfluorescence was related primarily to the size of the wheal, and therefore, to the quantity of wheal fluid. Injection of a nonwhealing material, as physiologic saline, produced some, although slight, increase in fluorescence, probably due to the increase in subepidermal fluid. The ragweed wheals, urticarial wheals, and areas of dermographism were hyperfluorescent for the same reason, adding to the view that these phenomena are associated with increased capillary dilatation and permeability, as in the histamine wheal.

Epinephrine acts to contract capillaries, empty them, decrease hydrostatic pressure, increase the thickness of the wall, and decrease permeability. The fluorescence curves for normal skin in patients given epinephrine were within normal limits. Perhaps under resting conditions imposed upon these patients,

few of the true capillaries were open and available for closure by the epinephrine. The epinephrine was effective in closing some of the capillaries in the histamine wheal, reducing wheal fluid, wheal size, and hyperfluorescence.

Pyribenzamine, a histamine antagonist which blocks the action of histamine, had no effect on the fluorescence over normal skin. In massive oral doses it reduced the hyperfluorescence over the histamine wheal. This was probably due to a decrease in size of the wheal, as further evidence of the antihistaminic action of Pyribenzamine.[14]

From the experiments, it appears that the capillaries of allergic individuals function normally, but we cannot say whether their structure is also normal. Evidence exists to suggest that the structure may not be normal. Redisch and Pelzer[15] reported abnormal nailfold capillaries in patients with migraine. In the few patients with urticaria we have observed, the nailfolds have an increased number of abnormal forms of capillaries. The capillaries in allergic patients react normally to histamine. They react further to specific antigens, probably following release of the H-substance, to form a wheal. It is this latter reaction which differentiates the allergic from the nonallergic patient.

SUMMARY

1. In this paper we have presented observations on fluorescence in allergic and nonallergic patients following intravenous administration of fluorescein and using the dermofluorometer under the following circumstances: (a) fluorescence in allergic and nonallergic patients over normal skin and wheals produced by histamine, ragweed extract, and physiologic saline solution; (b) fluorescence in urticarial wheals; and (c) fluorescence following administration of epinephrine and Pyribenzamine.

2. There is a narrow range of dermal fluorescence in normal subjects following intravenous injection of fluorescein.

3. The fluorescence curves of allergic patients fall within the normal range.

4. Histamine wheals show considerable hyperfluorescence which is of the same degree when histamine is injected in allergic and nonallergic patients.

5. The fluorescence of a wheal is related to size of the wheal rather than manner of production.

6. Epinephrine reduces fluorescence in histamine wheals.

7. Fluorescence curves for normal skin are not affected by epinephrine or Pyribenzamine.

8. Massive oral doses of Pyribenzamine appear to reduce fluorescence of the histamine wheal.

The authors wish to acknowledge their indebtedness to Miss Romayne A. Teufel, R.T. for her technical assistance in these experiments.

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DISCUSSION

ABRAHAM WALZER, Brooklyn, N. Y.—A slight degree of inflammation anywhere in the skin or mucous membrane leads, physiologically, to a pronounced hyperfluorescence immediately after the intravenous injection of a 5 per cent solution of fluorescein containing 5 per cent sodium bicarbonate. This has been shown to be due not to an increased capillary dilatation of the site, but mainly to an increase in the amount of fluid which develops locally as a result of an increased permeability of the vessels.

The experiments of Drs. Criep and Levine have corroborated this fact. They showed that wheals of any type, whether allergic, dermographic, or histamine induced, demonstrate a marked fluorescence, as compared to the adjacent skin; and the larger the wheal the greater the fluorescence. When the authors compared histamine wheals induced in allergic and in nonallergic subjects, they found the degree of fluorescence alike in both. They therefore justly concluded that the capillaries of allergic individuals show no greater permeability than the capillaries of nonallergic individuals.

Herrman and Kanof* have recently reported studies with the fluorescence technique and the dermofluorometer performed on wheals and in numerous acute and chronic dermatoses. Their experimental wheals were of two types, nonallergic induced with histamine and allergic induced with "protein" allergens. The fluorescence was apparently of the same degree in both types, since they make no mention of any differences noted. They found that the fluorescence in five cases of urticaria was more pronounced than in any of the dermatoses studied. But here again they report no differences in the degree of the reaction obtained in the various urticarias. The results reported by Drs. Criep and Levine, therefore, fit in very well with those of Herrman and Kanof. Apparently with this technique no characteristic differences may be detected in wheals in allergic and nonallergic subjects.

Herrman and Kanof further reported their fluorescence experiments in cases of allergic dermatoses, such as acute contact dermatitis and chronic atopic eczema, and in cases of nonatopic diseases such as second degree burns and circumscribed neurodermatitis. They found a uniform degree of fluorescence in all of these diseases. The brightness in the affected areas was usually proportional to the degree of localized superficial edema and was therefore more pronounced in lesions with vesiculation. They concluded that the type and degree of fluorescence are indications of accelerated extravasation, transudation, and resorption of fluid at the diseased sites. In other words, the degree of fluorescence was not closely correlated with allergic factors.

*J. Invest. Dermat. **7**: 210, 1946.

SENSITIVITY TO EDIBLE VEGETABLE OILS*

KARL D. FIGLEY, M.D., TOLEDO, OHIO

IT HAS long been known that the seeds of certain plants from which edible vegetables oils are obtained contain allergens which are often extremely potent. Examples are cottonseed, soya bean, peanut, and corn. In particular, cottonseed always attracted considerable attention from allergists because of the widespread use of its products and the severity of the allergic reactions in persons who are hypersensitive to certain proteins of the seed. From the seed embryo of the cotton plant, one or more water-soluble fractions of high allergenic potency can be extracted. This was proved in an outstanding piece of investigative work by Spies, Bernton, and Stevens,[1] who isolated from cottonseed an extremely potent water-soluble fraction, composed primarily of a protein and a polysaccharide. They called this fraction CS-1, and attributed its allergenic properties to the protein component. From CS-1, they also made a carbohydrate-free picrate with equally powerful allergenic properties.

Commercially, a highly refined, edible oil is obtained from cottonseed. This is used in salad and cooking oils, mayonnaise, salad dressings, oleomargarines. Plastic shortenings are made from it by hydrogenation, under trade names such as Crisco, Snowdrift, and Spry. Cotton seed oil is widely used in industry and to some extent for pharmaceuticals and cosmetics. From the "cake" which remains after the oil is expressed from the seeds, cottonseed meal is derived. Being rich in protein, this meal is extensively used as an ingredient of feeds for livestock. Formerly, cottonseed meal found much use as fertilizer, but its expense now largely prohibits this use. It is also used for human consumption as cottonseed flour by the baking industry, where it may be used in minor proportions in some brands of doughnuts, fig bars, and some cookies. Occasionally small amounts of the flour enter into the filling of chocolate coated candies. Diabetics have used cottonseed flour because of its high protein and low carbohydrate content. Thus, cottonseed meal or flour can be contacted by the unwary allergic individual, either through inhalation from feeds and fertilizers, or by ingestion of foods containing the flour.

Individuals specifically hypersensitive to cottonseed usually give immediate and violent allergic reactions either by inhalation or ingestion. There occur symptoms of allergic rhinitis; profuse sneezing, itching, coryza, and nasal obstruction, accompanied by lachrymation and suffusion of the conjunctivae. Severe asthma usually follows. Frequently, generalized urticaria and edematous swellings of the soft tissues form part of the picture. Gastrointestinal symptoms, such as severe cramps and diarrhea, may supervene particularly from ingestion of proteinaceous products of cottonseed. These severe reactions have impressed themselves on the allergist so that the literature contains many

*Presidential Address. Read at the Fifth Annual Meeting of the American Academy of Allergy, Atlantic City, N. J., Dec. 6-8, 1948.

warnings with regard to the danger of grave constitutional reactions from careless skin testing with cottonseed extracts. Authorities advise against intracutaneous testing. The CS-1 fraction isolated by Spies, Bernton, and Stevens is so potent that constitutional reactions may be induced by cutaneous tests with it, in dilutions less concentrated than 1:100,000.

In view of the highly allergenic nature of cottonseed, it is not surprising that allergists for the most part have made no distinction between sensitivity to the oil and to the meal. It has been assumed that the oil contains sufficient traces of the highly potent seed protein to cause symptoms such as are produced by ingestion or inhalation of the meal or flour. Therefore, when the allergist encounters a cottonseed-sensitive patient, he not only gives the patient instructions regarding the avoidance of cottonseed by inhalation and ingestion, but he gives meticulous instructions about avoiding the oil of cottonseed as it occurs in salad dressings, shortenings, oleomargarine, bakers' products, packed sardines and tuna fish, potato chips, etc. This prohibition of cottonseed oil consumption has two great disadvantages. In the first place, it is extremely difficult for the patient to conform to this regimen; in fact, it is practically impossible, since the oil enters into the composition of so many foods where its presence is unknown to the patient. Secondly, it greatly narrows the patient's diet range.

Bernton and his co-workers were the first publicly to question the assumption that cottonseed oil is a frequent and active sensitizing agent. In a most important paper[2] read before the American Society for the Study of Allergy in May, 1939, he and his associates effectively disproved this assumption. They summarized their observations and conclusions as follows: "By every available test we have been unable to demonstrate a correlation between cutaneous and clinical sensitiveness to the specific allergen of cottonseed, and clinical sensitiveness to edible cottonseed oil or to hydrogenated cottonseed oil. The evidence we have assembled proves that the specific and exceedingly potent water-soluble allergens of the cottonseed embryo do not occur in refined cottonseed oil. Therefore, patients who are clinically sensitive only to water-soluble cottonseed extractives may safely be spared the inconvenience of attempting to avoid foods containing cottonseed oil."

For some reason, this study by Bernton and his associates has been largely overlooked or ignored by allergists. Textbooks on allergy have appeared since the paper was published, and in them, as in the past, no distinction was made between sensitivity to the oil and to the water-soluble fractions of the cottonseed. One exception to this statement must be made: Unger, in his book, *Bronchial Asthma*,[3] does refer to Bernton's paper and discusses it in some detail.

Recently, this matter of alleged hypersensitivity to edible vegetable oils has taken on renewed interest and had attention focused on it by an unusual train of circumstances, the recital of which forms the *raison d'être* of this paper.

In the fall of 1947, The Federal Security Administrator instituted a public hearing in Washington[4] for the purpose of establishing definitions and stan-

dards for salad dressings. One problem considered was whether it was desirable that the labels of these foods indicate the particular vegetable oil, or oils, contained therein. The point at issue, to which the testimony of six allergists was directed, was whether the vegetable oils in mayonnaise, French dressings, and related salad dressings need be specifically identified for the protection of allergic consumers.

The first two medical witnesses at this hearing testified essentially that a considerable proportion of people hypersensitive to cottonseed protein are also allergic to cottonseed oil. One referred to a paper, "Cottonseed and Kapok Sensitization," he had published in 1929.[5] In this article he reviewed a series of thirteen patients who reacted allergically to cottonseed protein, from a group of 530 consecutive cases. The percentage of reactors was 2.4. Of these thirteen patients, he testified that five had allergic reactions from cottonseed oil "in some form." He deduced this from the fact that their symptoms cleared upon avoidance of cottonseed oil-containing foods. He had not, however, conducted a critical test by having these five patients ingest cottonseed oil on a fasting stomach, under observation. Both witnesses stated that some persons are allergic to soya bean oil, some to olive oil, some to peanut oil, some to corn oil, but that as a rule, people are not allergic to all vegetable oils, merely to a particular one.

The testimony of Dr. Harry S. Bernton of Washington, D. C., dealt largely with the findings reported in his article, "Significance of Cottonseed Sensitiveness,"[2] which has been summarized earlier in this paper. Dr. Bernton also referred to a survey of 1,069 patients in an allergy clinic, all tested with a water-soluble extract of cottonseed, which showed that 5.9 per cent reacted positively. From these positive reactors, he selected the five very sensitive cases for critical study which were discussed in detail in his article mentioned above. Dr. Bernton emphasized that the only reliable test for sensitivity to any edible vegetable oil is the ingestion of the oil on a fasting stomach. He further stated that sensitivity to the water-soluble extracts of a parent seed does not connote sensitivity to its oil. Finally, Dr. Bernton stated that while he had never encountered a proved case of vegetable oil sensitivity, he thought such might exist, and if so, the allergen would be other than a water-soluble protein fraction.

Dr. John H. Mitchell of Columbus, Ohio, was the next medical witness. He testified that he had recently reviewed a series of 906 asthma patients, all of whom had been tested with a water-soluble cottonseed extract. Thirteen (1.4 per cent) reacted positively. Of these thirteen, six were very sensitive to cottonseed protein. All six were given cutaneous (scratch) tests to cottonseed oil with negative results. All six were then given subcutaneous injections of from 0.5 to 1.0 c.c. of sterile cottonseed oil. There were no reactors. Then, under Dr. Mitchell's immediate observation, all six patients were fed cottonseed oil in doses of one-half ounce each. None reacted to this ingestion test. Dr. Mitchell stated in concluding his testimony that he had never encountered a case of authentic vegetable oil sensitivity.

I was privileged to appear at these hearings as a witness and was called to testify after Dr. Mitchell. I testified that I had reviewed a series of 1,301 allergy patients, all of whom had been tested for cottonseed sensitivity. Sixteen reacted significantly, and all had been shown to be clinically sensitive to cottonseed by inhalation or ingestion. The percentage of reactors was 1.23. Of these, seven were acutely sensitive to cottonseed protein. (Three of the seven had severe constitutional reactions by receiving scratch tests inadvertently performed with undiluted cottonseed fraction, CS-1). All seven gave negative scratch tests to cottonseed oil. Also, none evinced allergic reaction upon swallowing cottonseed oil in varying amounts. I then referred to an article I had published in 1942,[6] in which I reported two cases as being sensitive to some substance in cottonseed oil, other than cottonseed protein.

Case 1 was that of a man with so-called "intrinsic asthma," who gave negative skin tests to all common foods and inhalants, including cottonseed protein. Inhaling cottonseed meal caused no symptoms. A passive transfer test using the patient's serum was negative to cottonseed. Results of elimination diet and ingestion tests with cottonseed oil and other vegetable oils seemed to indicate sensitivity to cottonseed oil. This conclusion was stated in the published article.[6]

Case 2 was that of a woman, aged 44 years, whose various symptoms cleared following avoidance of all cottonseed oil products in her diet. She was skin tested with all the extracts used in the first case. The tests were all negative. Ingestion tests apparently proved cottonseed oil to be an offender.

I then testified to the subsequent history of these two patients. Case 1, seen again on Feb. 25, 1942, gave a negative intracutaneous test to cottonseed oil and showed no effect from swallowing a small amount of the potent cottonseed protein fraction, CS-1. He was then given twelve samples of hydrogenated oils, identified by number only. Six were cottonseed oil, with six soybean oil samples as controls. The patient was instructed to swallow one-half ounce or more of a single sample at intervals of one or two days when free from asthma. The oils were taken between February 27 and March 29, 1942, after which the patient reported by letter. Asthma symptoms followed ingestion of one of the six cottonseed oil samples and one of the six soybean samples, with negative results in all other trials. Thus the patient failed to react to hydrogenated cottonseed oil five times out of six trials.

With reference to Case 2, I stated that I last saw this patient in September, 1941, at the time of her original ingestion tests. She was contacted by telephone on Dec. 26, 1947, and it was learned that she was using cottonseed oil products with no ill effect. The patient also stated she had discovered that milk ingestion provoked many of the symptoms originally complained of.

In view of the lack of clear-cut findings with respect to these two patients, I testified that I was obliged to change my original opinion that they represented proved cases of sensitivity to cottonseed oil. It was amply proved that they were not allergic to cottonseed protein.

Dr. Mary H. Loveless, of New York City, testified that she reviewed the records of many persons who had been tested for cottonseed protein sensitivity at an outpatient allergy clinic in New York City. Only one case proved clinically sensitive. This patient had an extremely severe allergic reaction, with collapse, from ingesting a piece of candy which was found to contain a small amount of cottonseed flour. The patient gave a strongly positive skin test to water-soluble cottonseed extract, but had no reaction to the ingestion of cottonseed oil, as a direct test. Dr. Loveless stated that at the Annual Meeting of The American Academy of Allergy held in St. Louis, December, 1947, she interrogated twenty-three well-known allergists. Not one knew of a proved case of edible vegetable oil sensitivity. She emphasized in her testimony that the only reliable test for vegetable oil sensitivity is to have the patient eliminate the suspected oil from his diet for several days, and then on a fasting stomach, ingest the oil. The test should be repeated several times, and psychogenic factors should be avoided by making the identity of the oil.

My survey of the testimony pertaining to vegetable oil hypersensitivity would be incomplete without mention of relevant information developed in the testimony of two vegetable oil technologists, Mr. J. Lester Crain and Mr. Albert F. Sanchez. Their testimony concerned industrial practices involved in production and utilization of edible oils. I refer you to the official transcript of the hearing for detailed evidence supporting these pertinent facts: Admixture of one kind of edible oil with another is inevitable at any one of several stages of transportation and processing oilseeds and oils, before the finished product is delivered to the manufacturer of salad dressing. The degree of admixture is variable and presumably cumulative. However, the maximum degree of admixture is not within the range of detection by analytical methods; that is, 0.2 per cent. The matter of specific identification of the kind of oil in a salad dressing would be further complicated by changes from one kind of oil to another, or from a single kind of oil to a mixture. Such changes may be necessitated one or more times in the course of one day to keep the plant in operation. Conceivably, labeling could conform to those changes, but would add to the cost of the product.

The testimony alone of the medical witnesses at this hearing covered several hundred pages in the reporter's transcript. The information given above represents the content of their testimony. One question which seemed to be prominent in the mind of the Government attorney was why have numerous competent allergists, as represented in the literature, thought they were dealing with cases of actual vegetable oil sensitivity. The answer is, as brought out by the last four medical witnesses, that sensitivity to the water-soluble protein fractions of the seed has been assumed to imply, but does not actually denote, sensitivity to the oil. These witnesses also showed that if a vegetable oil should be allergenic in a given instance, its ingestion would be followed by unmistakable allergic symptoms. It was pointed out in their testimony that symptoms of fat intolerance could follow the ingestion of appreciable quantities of the oil, but that these symptoms are not allergic in nature.

Recently, Dr. Alan G. Cazort[7] has made a valuable contribution to the matter under discussion, in the form of two case reports. Case 1 was a medical student whom Dr. Cazort found to be clinically sensitive to corn meal by inhalation and ingestion. The patient gave a strongly positive scratch test to corn meal, but negative scratch tests with cornstarch and corn syrup. No test was made with corn oil by scratch. After having avoided contact with any known corn products for five days, the patient was fed one ounce of refined corn syrup. No reaction whatsoever ensued. A few days later he ate freely of cornstarch pudding without symptoms resulting. After that, he drank an ounce of corn oil without symptoms. One-half teaspoonful of raw cornstarch put inside the patient's lip next to his gums, caused no reaction after one-half hour. Dr. Cazort stated, "This confirms my conclusions from similar experiments several years ago that any corn antigen in corn oil, cornstarch, or corn syrup is negligible clinically."

Dr. Cazort's second case report[7] concerned a boy, aged 3 years, who was known to be quite acutely sensitive to peanuts, and who gave a positive scratch test to water-soluble peanut extract. A scratch test with peanut oil was negative. Under Dr. Cazort's direct observation this boy swallowed varying amounts of Planter's peanut oil, with no ill effect whatsoever.

Since the processing of vegetable oils seems likely to affect their allergenic activity, Dr. A. S. Richardson,[8] Associate Director of Chemical Division, The Procter and Gamble Company, was asked to comment on this subject. His reply is quoted below:

"The principal vegetable oils for edible use in the United States are cottonseed oil and soybean oil, the average annual production of each being slightly more than 1,200,000,000 pounds during the years 1942-6. In a typical year, the amount of either of these oils produced in this country is about twice the total of all other edible oils produced or imported. Corn oil and peanut oil are next in importance in the order named, but before World War II coconut oil was third in importance for edible use.

"The vegetable oils are obtained either by applying pressure to oil-bearing seed or by extracting with solvent. Hydraulic presses are in widespread use, and there is also considerable continuous pressing in screw presses, commonly known as expellers. Solvent extraction, long used in Europe, has been developed in this country mainly during the past 20 years for producing oil from a greatly increased domestic crop of soybeans. In the present discussion, we can best ignore most of the endless variations in the processing steps required for carrying out these methods of producing vegetable oil. In each such method there are only two process variables which seem likely to affect the allergen content of an edible oil; namely, heat treatment and mechanical separation.

"In preparation for either method of pressing, the seed material is heated to temperatures ranging from about 212° F. to about 270° F., and a still higher maximum temperature, probably about 300° F., prevails for a brief time inside the expeller as it is commonly used for manufacture of soybean oil. The temperature of the crude oil coming from the presses is nearly always held below

212° F. In solvent extraction practice, there is on the average much less heating of oil, and of seed material prior to removal of oil. In view of these variations in manufacturing methods, it seems unlikely that heat treatment during production of vegetable oils could be depended upon to destroy active allergens, if any, which such oils might contain.

"Oil produced by pressing usually undergoes extensive settling, and is also occasionally filtered through heavy, closely woven cloth. The requirements of the solvent extraction process are more exacting with respect to removal of suspended solids from the oil, which is filtered while still dissolved in the solvent. The removal of protein and other suspended material by settling or filtering the oil is quite efficient as judged by ordinary standards, but not necessarily efficient as an allergist interested in trace contamination might decide the matter.

"In order to convert the crude vegetable oils (with the minor exceptions of olive oil and cocoa butter) to acceptable edible products, at least three purification treatments are required; namely, alkali refining, filtering, and steam deodorizing. Also water washing, treatment with bleaching clay, and hydrogenation are widely used optional steps in the production of edible vegetable oils in final form.

"Water washing of fatty oils is used in various stages of processing. In the so-called 'degumming' process, crude soybean oil is mixed with a very small amount of water, and the mixture centrifuged. Traces of soap in refined oil are sometimes removed by mixing water into the oil and then allowing it to settle out by gravity. Whatever its purpose, water washing of an oil is a treatment inherently likely to remove an allergen of protein nature, if present.

"Alkali refining is the first essential step in the production of edible products from crude cottonseed, soybean, and similar oils; and it is incidentally also a water washing of the oil. The oil is mixed with an aqueous solution of sodium hydroxide, which reacts rapidly with any free fatty acid present, more slowly with a little of the oil itself, to form soap. The treatment also serves to coagulate lecithin-like material and other impurities in the oil. The mixture is settled or centrifuged to separate the purified oil from a lower layer called 'foots,' containing water, soap, and coagulated material. As far as the writer knows, protein has never been identified as a component of an alkali refined vegetable oil, even by qualitative test.

"Filtering and treatment with bleaching clay can best be considered together. Most of the refined vegetable oil, either freshly prepared or from storage, is subjected to 'bleaching.' The oil is mixed with a small percentage of a clay which has the property of decolorizing the oil by adsorption. Sometimes a still smaller quantity of bleaching carbon is also used. The temperature is usually in the range 160-240° F. The clay and carbon are removed by use of a filter press. Even if no improvement in color of the oil is wanted, the refined oil is invariably filtered, nearly always with the use of enough bleaching clay to act as a filter-aid. With or without the full bleaching treatment, such a filtration serves to remove any of the 'foots' layer left in the refined oil due to imperfect settling or centrifuging.

"Hydrogenation of vegetable oil consists in combining hydrogen with unsaturated fatty glycerides in the presence of nickel as catalyst. The process is used for making two types of plastic shortening. There is the all-hydrogenated shortening (including the two most highly advertised brands of vegetable shortening), which is prepared by subjecting all of the oil to a limited hydrogenation, mainly for the purpose of improving keeping quality. 'Compound' or 'standard shortening,' so-called (in the trade), is made by almost completely hydrogenating a part of the oil and mixing the resulting hard fat with, for example, about 7 times its own weight of unhydrogenated oil.

"Oils are usually refined, treated with bleaching earth and filtered before they are hydrogenated. Conditions of hydrogenation vary and are more shrouded in secrecy than other processing details, but the writer ventures the guess that maximum temperature for hydrogenating edible oils is nearly always in the range 250-350° F. Hydrogenation is followed by another filtration of the oil, frequently in combination with use of bleaching earth. It would be difficult to suggest a series of treatments more likely to yield an oil free from the seed protein.

"Steam deodorization is the final treatment shared by practically all edible oils. This is simply a vacuum steam distillation to remove the volatile products which contribute an undesired flavor. In a typical example, the oil is maintained at 400° F. under ½ inch (mercury) pressure for 3 to 4 hours, during which time steam is blown through the oil at the rate of .03 lb. per pound of oil per hour. Most of the edible vegetable oil in this country is deodorized at a temperature not less than about 390° F., and probably a negligible amount of it is deodorized at any temperature much below 366° F. (the temperature of steam at 150 pounds pressure). Dr. George S. Jamieson in his well-known monograph, 'Vegetable Fats and Oils,' states that deodorization temperature is usually from 200° to 225° C. (392° – 437° F.). The temperatures thus used in deodorization are sufficient to denature almost any protein material, and it will indeed be surprising if any allergen of protein nature can retain its activity after such treatment.

"If there are active allergens in the vegetable oil manufactured and sold for edible use in this country, a chemist's best guess is that they are entirely different from the allergens commonly associated with the protein of the oil-bearing seeds and studied in the form of aqueous extracts prepared at low temperature."

The main purpose of this presentation is to call attention to a unique contribution to the literature of allergy in the form of a federal, public hearing devoted in part to the subject of edible vegetable oil sensitivity. The sworn testimony of the medical witnesses is a matter of record. Then too, it is hoped that this presentation will inspire further critical search to settle once and for all the question of allergic sensitivity to edible vegetable oils. At some future meeting of this society, a symposium on this subject might profitably be held.

SUMMARY

As a result of this hearing it was brought out:

1. That allergic sensitivity to the water-soluble protein fractions of various seeds from which edible vegetable oils are obtained is fairly frequent and severe, especially with reference to cottonseed. However, allergic sensitivity to the water-soluble proteins of a seed does not indicate allergic sensitivity to the refined oils expressed from that seed.

2. To date, there have been no cases of edible vegetable oil sensitivity reported in the literature, periodical or textbook, for which authenticating evidence is presented.

3. If allergic sensitivity to refined edible vegetable oils exists, the causative factor must be some substance other than the water-soluble proteins of the parent seed.

4. The only reliable test for sensitivity to refined vegetable oils is the ingestion test. This should be conducted under conditions to minimize the influence of anticipation or suggestion on both the observer and the subject.

5. The methods of processing edible vegetable oils render their retention of any allergenic activity highly unlikely.

ADDENDUM

Since this paper was written, the testimony of the allergists at the federal hearing has been compiled in book form by The National Cottonseed Products Association. The title is *Allergy to Cottonseed and Other Oilseeds.*

A copy of this book is being presented to the library of each medical school in the United States, to several of the Canadian medical schools, and to a number of medical and scientific research libraries of the United States and Canada. None of this edition of 300 copies is available for purchase or for general distribution otherwise.

The object of the distribution is to ensure continuing accessibility of the book by loan from these libraries, or through other libraries by inter-library loan.

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   (A transcript of the testimony given at this hearing relating to vegetable oil sensitivity is on file in the Library of the U. S. Department of Agriculture, Washington, D. C. It is in four volumes, any or all of which may be borrowed from the Department of Agriculture Library by applying through one's local library on the basis of inter-library loan.)
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A STATISTICAL STUDY OF THE EMOTIONAL COMPONENT IN ALLERGY: ITS RELATIONSHIP TO THE AGE AND SEX DISTRIBUTION OF 5,583 PATIENTS*

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WE HAVE studied the age-sex distribution in 5,583 private patients with allergic conditions.

For the study, we used the age of the patient when first seen in our office instead of the actual age of onset, since the latter is very difficult to obtain with exactitude. Our first analysis (Table I) showed that between the ages of 0 and 12, inclusively, boys were affected significantly more frequently than girls. There were 831 boys (65.1 per cent) and 425 girls (34.9 per cent). Between the ages of 12 and 20, inclusively, there were 335 boys and 336 girls, i.e., no significant difference in sex distribution. When the figures for the 20- to 30- and 30- to 40-year groups were compared, the sex distribution was predominantly in favor of the women. There were 401 (34.2 per cent men and 764 (65.8 per cent) women in the 20- to 30-year group, and 447 men (42.7 per cent) and 600 women (57.3 per cent) in the 30- to 40-year group. There were no significant differences between men and women in the age groups above 40. Consideration of the entire 5,583 cases as a whole, showed a relatively even distribution between the sexes.

TABLE I. SEX DISTRIBUTION OF 5,583 PATIENTS BY AGE GROUPS (COHEN)

	0-12	12-20	20-30	30-40	40+	TOTAL
♂	831	335	401	447	676	2,690
♀	425	336	764	600	768	2,893

Because it seemed desirable to have more definite information concerning the exact age of the shifts in preponderance noted above, we analyzed six groups each consisting of approximately 500 cases chosen at random and classified by 5-year age differences (Table II). Analysis of the data in this table shows a distribution similar to that in Table I with the males predominating between the ages of 0 and 14, inclusively, and the women between the ages of 20 and 40.

During the past several years, we have been attempting to assess the emotional components in allergy. Our clinical experiences gave us the impression that this component was of more importance in women than in men.

Since clinical impressions are not always borne out by careful study of the facts, we analyzed the records of 662 private patients seen during the year 1947.

All these patients were seen at frequent intervals during a period of at least one year. All of them had respiratory symptoms. Some had dermatitis,

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migraine, gastrointestinal symptoms, and manifestations of allergy in other systems in addition. Each was thoroughly studied as a problem in internal medicine, and from the first contact throughout the entire management, attempts were made to elicit not only the immunological factors, but also the various nonspecific ones which so often play a part in these problems.

TABLE II. SEX DISTRIBUTION OF 3,150 PATIENTS BY FIVE-YEAR AGE GROUPS (COHEN)

	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65 +	TOTAL
♂	119	247	173	105	94	123	113	112	119	85	93	67	31	26	1,537
♀	95	118	94	136	167	210	196	163	121	108	83	64	29	29	1,613
♂	569				442				421						
♀	307				736				434						

Just as we used clinical methods to determine the presence or absence of organic complications whether cardiac, vascular, or pulmonary, we used the Cornel Index, the social history, and conferences with the patients to evaluate the personality.

All the information obtained by immunological or psychological methods was verified in terms of etiology much as one establishes the significance of a positive skin reaction, namely, by exposing a patient to contact with the suspected excitant.

The 662 cases were first divided into those who showed evidences of extrinsic allergic disease including positive skin or mucous membrane reactions, and second, into those in whom no specific etiological factor could be determined even after environmental control of contact with inhalants and the use of experimental diets.

All of the cases classified as extrinsic were managed by immunological methods to the best of our ability. While such treatment was being carried out, we attempted to find the causes for any exacerbation of symptoms while continuing to study the personality of the patient.

It soon became apparent that there were patients (Group I) in whom all symptoms could be explained by allergen contact, exposure to irritant fumes, or weather changes, regardless of whether or not the patient had a neurosis. In other words, any existing neurosis played no part in the production of allergic symptoms.

In contrast to patients in Group I were others with extrinsic allergic respiratory disease, whose attacks could not be explained entirely by allergen contacts, nonspecific irritation, or weather changes. These form Group II, and we were ultimately able to demonstrate in them the presence of emotional trigger mechanisms which set off reactions in shock organs previously conditioned by allergic reactions.

Those patients who could not be classified as extrinsic were easily divided into Group III, those with organic changes such as nasal and sinus polyposis, emphysema, and pulmonary hypertension of various degrees, and Group IV,

those with the typical symptomatology of nasal or bronchial allergic disease without the pathological changes of allergy.

In the cases in Group IV, like those in Group II, there were demonstrable psychic influences which were of etiological importance. Group IV cases differ from those in Group II only in that the primary cause is not allergic, but psychogenic, i.e., any conditioning of the shock organ which may be present is not due to a demonstrable allergic mechanism.

Table III shows that the distribution of these 662 cases by sex and age follows the pattern of the whole group with the men approximately twice as frequent as the women, between 0 and 15 years of age and the women twice as frequent as the men between 20 and 40 years of age.

When the 662 cases were classified into the four groups just described (Table IV) it became apparent that the increase in women between the ages of 20 and 40 was due to the very great preponderance (approximately 9:1) of women of that age distribution in Group II. If one removes the figures for the Group II cases from the total figures in Table III, one finds (Table V) that men still maintain a 2 to 1 frequency over women from 0 to 15 years of age, but that there is now no significant differences between men and women in the 20- to 40-age group.

TABLE III. SEX AND AGE DISTRIBUTION OF 662 PATIENTS MANAGED DURING 1947

	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65+
♂	47	80	27	27	17	24	21	10	19	9	16	15	8	1
♀	24	30	22	35	35	29	46	24	28	25	12	12	2	3
♂	154				72				67					
♀	76				134				82					

TABLE IV. SEX AND AGE DISTRIBUTION OF 662 PATIENTS DIVIDED INTO FOUR ETIOLOGICAL GROUPS

	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65+
							Group I							
♂	45	71	26	23	17	20	19	18	15	6	10	7	3	1
♀	20	25	18	29	26	16	26	17	19	19	6	7	1	2
♂	142				87									
♀	92				104									
							Group II							
♂	2	9	1	4	0	1	1	1	0	1	2	0	0	0
♀	3	5	4	8	9	12	19	13	6	2	0	0	0	1
♂	12				7									
♀	12				61									
							Group III							
♂	0	0	0	0	0	1	0	1	4	1	4	5	5	0
♀	1	0	0	0	0	0	0	1	2	0	5	4	1	0
							Group IV							
♂	0	0	0	0	0	2	1	0	0	1	0	1	0	0
♀	0	0	0	2	0	1	1	3	1	4	2	1	0	0

TABLE V. AGE AND SEX DISTRIBUTION OF 662 PATIENTS WITH THOSE CLASSIFIED AS GROUP II AND GROUP IV REMOVED

	0 - 15	20 - 40
♂	132	62
♀	64	68

DISCUSSION

These observations confirm the studies of Nelson[1] and the statement of Bray[2] that "practically all allergic conditions are more common in males than in females from birth to fifteen years and in females than in males from the ages of fifteen to forty-five." Both Bray and Nelson attribute these distribution differences to some unknown factors associated with puberty.

Our study indicates that emotional disturbances which initiate attacks are found much more frequently in women than in men especially between the ages of 20 and 40. In our series of patients this increased frequency accounts for the 2:1 ratio of women to men shown at these ages by the entire group. The study further points out the importance of the recognition and management of the emotional component in patients seen in allergists' offices.

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ALLERGY SESSION PROGRAM

American Medical Association Meeting

Atlantic City, N. J., June, 1949

1. Asthma in Children—Salient Diagnostic Problems
   Speaker: Bret Ratner, M.D., New York, N. Y.
   Discussor: James C. Overall, M.D., Nashville, Tenn.
2. Rational of Therapy With Bacterial Vaccines in Allergic Diseases
   Speaker: Oscar Swineford, Jr., M.D., Charlottesville, Va.
   Discussor: Walter S. Burrage, M.D., Boston, Mass.
3. Status Asthmaticus
   Speaker: George Piness, M.D., Los Angeles, Calif.
   Discussor: Maurice S. Segal, M.D., Boston, Mass.
4. Provocative Diets in the Recognition of Food Allergy
   Speaker: Howard J. Lee, M.D., Milwaukee, Wis.
   Discussor: Hal McC. Davison, M.D., Atlanta, Ga.
5. Dermatoses Which May Have an Allergic Etiology
   Speaker: James R. Webster, M.D., Chicago, Ill.
   Discussor: Nelson Paul Anderson, M.D., Los Angeles, Calif.
6. The Clinical Indications and Limitations of the Antihistaminic Drugs in Allergy
   Speaker: Walter L. Winkenwerder, M.D., Baltimore, Md.
   Discussor: J. Harvey Black, M.D., Dallas, Texas.
7. Chairman's Address: Clinical Experiences With the Diagnosis of Asthma
   Chairman: Charles H. Eyermann, M.D., St. Louis, Mo.

Original Articles

TRANSITORY PULMONARY INFILTRATIONS (LOEFFLER'S SYNDROME) WITH CASE REPORT*

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THE accidental finding of a migratory pulmonary infiltration associated with apparent cavitation in the lung field in an asthmatic during the ragweed pollination period prompted a review of the literature pertaining to Loeffler's syndrome. Amberson,[1] in 1924, discussed the question of annular shadows appearing in roentgenograms of the pulmonary fields and stated that such shadows may be pleuritic in origin rather than true cavities within the lungs. Arnell[2] reported the first authentic case of annular shadows caused by subpleural emphysema in a man suffering from asthma. The association of apparent cavitation in the lungs during an episode of pulmonary infiltrations of a transitory nature has been previously reported.[3]

Transient pulmonary infiltrations with eosinophilia during asthmatic episodes have been reported by others.[4-8] It is apparent from a study of these cases that this type of pulmonary reaction is encountered mostly in allergic individuals. In the main, the implications are that many of the cases described under such designations as eosinophilic pneumonia, Loeffler's pneumonia, etc., are instances of Loeffler's syndrome. Little has been added to Loeffler's concept other than a descriptive terminology.

The available reports of Loeffler's syndrome were studied with the view of ascertaining the incidence of asthma and the possible related causative factors, and the applicability of Loeffler's criteria as regards diagnosis. It was thought that because of the recent awareness of this type of tissue response in the lung and the prevalent-mass roentgenologic examinations, the percentage of reported cases would be high.

From the very beginning attention has been called to the relationship of such allergic manifestations as hay fever, asthma, vasomotor rhinitis, urticaria, and eczema to the migratory pulmonary infiltrations observed in Loeffler's syndrome. Maier[8] reviewed 100 cases at Loeffler's clinic and found that fifty-two demonstrated, either before or at the time of the pulmonary findings, one of the following: eczema, hay fever, migraine, vasomotor rhinitis, and urticaria.

The presence of transitory pulmonary infiltrations in an asthmatic during the ragweed pollination period, as in the case reported here, may be more than mere coincidence. It is recognized that Loeffler's syndrome may occur under many allergic conditions. It is reasonable to assume that in the asthmatic one may expect a high incidence of transitory pulmonary infiltrations. Yet, there are few reports available regarding the incidence of such pulmonary infiltrations

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in asthmatics. Breton[9] is credited with the statement that Loeffler's syndrome occurs in 5 to 8 per cent of asthmatics. Wright and his associates[10] made routine chest roentgenograms on 268 consecutive asthmatics. Twenty-four or 9 per cent had pulmonary consolidation; they were divided into two groups using the clinical presence or absence of fever and systemic illness as the basis for the separation. Fourteen or 5.3 per cent were classified as probable allergic consolidation and ten or 3.7 per cent as atypical virus pneumonia. The authors pointed out that the group classified as allergic consolidation did not meet the accepted diagnostic criteria as regards Loeffler's syndrome, because the consolidations were not migratory and the peripheral eosinophilia did not exceed that found in asthmatics without pulmonary infiltration. Saupe[55] observed pulmonary infiltrations in 11.6 per cent of 355 asthmatics.

Loeffler's[12] first five cases showed transitory roentgenological lung shadows, high blood eosinophilia, and a paucity of symptoms and clinical signs. Within four years of the first report, ninety-five additional cases were in the literature. Routine roentgenoscopy and roentgenography were responsible for a high percentage of these discovered cases.

Despite the expectancy of increased reports, the incidence of Loeffler's syndrome in the last ten years in no way parallels the percentage reported in the first five years. Indeed, as will be shown, many cases labeled Loeffler's syndrome do not adhere to the criteria of Loeffler. It is this disparity and apparent broadening of the concept of Loeffler's syndrome that speaks against the syndrome being a distinct entity.

The syndrome is characterized roentgenologically by pulmonary shadows which develop rapidly and whose disappearance may be as rapid. The infiltrations vary in density and extent and may occur anywhere in the pulmonary area. Loeffler emphasized the fleeting nature of the lesions and stated that the continuance of any individual shadow for more than a fortnight was evidence against the assumption that such findings were those of the syndrome.

Patients with the lung findings described are not very ill. Elevation of temperature may occur, but usually the course is afebrile. The physical findings are minimal. Some report such auscultatory changes as slightly increased resonance, or impaired percussion note, and roughened breath sounds with varying types of râles. In the asthmatic, the usual pulmonary sounds are not indicative of any infiltrative process; the unusual finding of pulmonary shadows is accidentally discovered in the course of roentgenographic examination or is suspected because of a pronounced increase in the blood eosinophilia.

Hematologic studies reveal a normal to moderately elevated leucocyte count and a pronounced increase in eosinophiles; this increase does not parallel the size of the fluctuations of the pulmonary infiltrations. The sedimentation rate is increased in most of the cases.

The diagnostic criteria in Loeffler's[23] syndrome are as follows; (1) the benign course and paucity of general findings, (2) the shifting pulmonary roentgenographic changes and the short duration of the pulmonary infiltrations, and (3) the concomitant but variable increase in blood eosinophiles.

At least two of the features mentioned occur in other disease entities. In periarteritis nodosa and tropical eosinophilia, high eosinophilic blood levels are

evident. In both conditions the roentgenograms of the chest may show distinct, disseminated mottling of the lungs and the physical findings in the chest may be like those of bronchial asthma.

Many authors[4, 13, 14, 15, 16] have described cases in which chronicity was the outstanding feature, the pulmonary infiltrations being present for many weeks or months associated with severe clinical symptoms. It[17] has been suggested that pulmonary infiltrations of the nature described should be termed eosinophilic pneumonitis. The variability both in pulmonary findings and clinical symptoms has led Kartagener[13] to suggest the following classifications; (1) Loeffler type, an acute process of short duration, (2) Loehr and Leon-Kindberg[18] group in which the acute symptoms are severe and septiclike, with persistence of the pulmonary infiltrations, and (3) the type reported by Kartagener in which the symptoms are mild but prolonged.

Rackemann[19] stated that if Loeffler's syndrome, intrinsic asthma, and periarteritis nodosa would respond to arsenic therapy as does tropical eosinophilia, then one may regard the four conditions as variations of the same fundamental disease.

Such classification, either on a therapeutic or symptomatic basis, is tenuous.

ETIOLOGY

Despite the general feeling that the syndrome is an allergic phenomenon, there is no unanimity of opinion as to the exact etiology. Many authors stress the association of the syndrome with bronchial asthma and other allergic manifestations. Loeffler and others believed that some of the cases were related to tuberculosis. However, the lack of calcification, the benign course, and the nontuberculous outcome speak against such an etiology.

Eosinophilia is commonly noted in various metazoal parasitic infestations. Many cases of children showing transient pulmonary infiltrations associated with intestinal parasitic infestation and high blood eosinophilia lend support to the opinion that helminthiasis might be an etiologic factor.[20-22]

Patients infected with Amoeba,[20] Ascaris,[28] *Ancylostoma braziliense*,[24] *necator Americanus*,[25] Strongyloides,[26] and *Trichinella spiralis*[21] exhibited transitory pulmonary shadows and an associated eosinophilia.

Wright and Gold[24] observed seventy-six cases of creeping eruption with classic cutaneous lesions. Of fifty-two of these cases studied for fourteen days or more, twenty-six developed transitory, migratory, pulmonary infiltrations and peripheral eosinophilia. In twenty-three of the twenty-six cases, positive skin tests were obtained with at least one of the extracts, ascaris and trichina, used for intradermal testing. According to Zweifel,[27] 40 per cent of the normal and allergic individuals, on testing with ascaris extract, will give positive reactions. He obtained positive reactions to ascaris extract in 70 per cent of cases with Loeffler's syndrome.

Engel[29] reported the presence of coryza, transitory lung shadows, and blood eosinophilia in individuals during the period of privet pollination in May. This constitutes the first evidence implicating a pollen as a causative factor. Alpher[30] observed the syndrome in a ragweed sensitive patient who developed a cough soon after the onset of the pollination period.

PATHOLOGY

The transient nature of the syndrome and its benignity has not afforded the pathologist much opportunity for study of the lesions in the lung. Although von Meyenburg[31] was the first to report pathological findings in four cases, these never did exhibit the transient nature of the syndrome. The pathological lesions were essentially those of lobular pneumonia in which the cellular portion of the exudate consisted largely of eosinophiles.

Herbut and Kinsey[32] attempted to follow the allergic phenomena in animals roentgenographically and to correlate these observations with the clinical manifestations. They succeeded in duplicating in rabbits the syndrome as observed in man. The lung shadows in the rabbits, however, were not migratory; the areas of lung involved developed to a maximum reaction and then cleared without reappearing or shifting. No eosinophilia was noted. The authors found hyalinization of the basement membrane of the mucosa of the bronchi together with edema and eosinophilic infiltration of the submucosa, along with congestion, edema, atelectasis, emphysema, and eosinophilic pneumonia of the lung proper.

Bayley[33] and associates reported one case, a 59-year-old woman who had had asthma for seven years. The abnormal roentgenographic findings regressed after seven weeks. Histologic examination of the pulmonary lesions revealed an unusual form of organized pneumonia in which there was an abnormally large number of eosinophilic leucocytes. Hypertrophy of the muscle of the bronchial wall was not pronounced, the basement membrane showed marked hyalinization and thickening. There were vascular changes consisting of hypertrophy of the media, and thickening of the intima of the arterioles and the small- and medium-sized arteries. The same vascular changes were reported by Harkavy in four cases of severe bronchial asthma.

Bergstrand,[15] in a recent article, discussed the morphologic similarities of lesions presumably allergic in nature. He reported four cases of asthma with transient infiltrations and blood eosinophilia. Necropsy examination revealed focal vascular changes in the lung, along with interstitial inflammatory change, serous exudate, eosinophiles, and giant cells.

THE INCIDENCE OF ASTHMA AMONG THE CASES REVIEWED

Among the thirty-six case reports reviewed, a diagnosis of bronchial asthma was established in twenty-five patients, an incidence of 69 per cent (Table I). The bronchial asthma was evident either previous to, or associated with, the finding of the transitory pulmonary infiltration. Eleven of the twenty-five cases with bronchial asthma were in females.

Positive cutaneous reactions to foods and inhalants were reported in fourteen of the thirty-six cases. Although the antigens mentioned were known to play a part in the allergic history, it was not possible to establish even a casual relationship between the supposed sensitivities and the presence of the transitory pulmonary infiltrations. Alpher's report and the case reported here are the only instances in which one may conjecture that the ragweed pollen was the causative agent as regards the pulmonary findings.

The ages of the patients in the cases reviewed ranged from 5 months to 63 years. Thirteen of the patients were in the third decade of life, and there was an almost equal distribution of the remaining cases in the other age groups.

In the group of thirty-six cases, a history of hay fever was present in five, in three of these there was also asthma. Such manifestations as eczema and gastrointestinal allergy were also noted in a few cases.

Only 25 per cent of the cases reviewed met the diagnostic criteria as regards Loeffler's syndrome. The remaining cases showed either recurrences of the pul-

TABLE I

AUTHORS	SEX	AGE	F. H.	ASTHMA	ALLERGENS	LUNG FINDINGS	LOEFFLER'S CRITERIA
Spector[41]	M	44	+			Prolonged	–
Hennell and Sussman[42]	F	21		+	Foods Pollens	Recurrence	–
	F	28		+	Foods Pollens		
	M	27		+			
	F	19		+			–
	F	26		+			–
Peabody[43]	M	21		+		Recurrence Prolonged	–
Serra[44]	F	44				Recurrence	
Eichwald and Singletary[45]	M F	49 59		 +			
Pirkle and Davin[46]	F	54				Prolonged	–
Sherlis[47]	M	24					+
Elkeles and Butler[3]	M	19		(H. F.)	Feathers, Grasses	Prolonged	–
Henderson and Peirce[48]	M	34	+	+			–
Peirce[11]	F	14		+	Ragweed		+
Crutchlow	M	54		+	Grass Ragweed		
Henderson	M	53		+			
McKay	M	63					+
Baer[5]	F	30		+			+
Smith[7]	F	55		+			
Ryan[49]	F	35					+
Gottlieb and Sharlin[50]	F	44		+ (H. F.)	Foods		+
Squier[51]	M	27		+ (Ecz.)	Danders, Dust, Pollens		+
	M	4		+ (Ecz.)	Dust, Pollens	Recurrence	+
	M	31		+ (H. F.)	Pollens	Recurrence	–
Gottdiener[52]	M	21		+	Danders, Dust		
	M	24	+	+ (H. F.)			
Hansen-Pruss and Goodman[6]	F	20	+	+			–
	M	22	+	+			
	F	35		+	Dust, Pollens		
	M	1		+			
	F	29		+			
O'Bryne[53]	F	5 mos.	+	Asthma later	Foods		–
Hendron[54]	F	46				Prolonged	
Alpher[30]	M	24	+	+	Dust, Foods	Recurrence	
	F	14	+	(H. F.)	Ragweed		+

monary infiltrations, months to years later, or there was present a prolongation of the pulmonary findings beyond the arbitrary limits of a month. Six of the cases had recurrences of the transitory pulmonary findings. These recurrences appeared in periods ranging from months to as long as six years. Some cases had more than one recurrence.

CASE REPORT

The patient, a white male of 16 years, was first seen on Sept. 15, 1947, because of asthma and hay fever symptoms. The asthma was of ten years' duration, being present throughout the year with exacerbation of symptoms during the ragweed season, and also in association with upper respiratory infections. Both the patient's mother and younger brother had asthma.

The first episode of asthma was observed in the patient when he was 6 years old. The attack followed an illness that had been diagnosed as influenza. At the age of 7, the patient was tested by an allergist and the following antigens gave "positive reactions"; wheat, milk, chocolate, Johnson grass, cedar, and ragweed.

The patient was a thin 16-year-old white male whose weight was 86½ pounds. Nasal examination revealed the mucosa to be pale and markedly boggy. On examination of the chest, there was hyperresonance to percussion over the bases; expiration was prolonged. There were scattered sibilant râles.

Intracutaneous testing showed marked reactions to house dust, ragweed (.01 mg. N), and moderate reactions to chocolate, corn, lima bean, and cedar. The response to ascaris extract was negative. He had an eosinophilia of 33 per cent on his initial visit. The laboratory findings are incorporated in Table II.

TABLE II. LABORATORY FINDINGS

	TOTAL WHITE	SEGS.	BANDS.	MONO.	LYMPHS.	EOSIN.	BASO.	SED. RATE	SPUTUM TBC.	SPUTUM EOSIN
9/15/47	12,500	50	1	7	8	33	1	7		
10/ 1/47								31		
10/ 8/47								22		
10/10/47									Neg.	8%
10/12/47									24 hr. conc. Neg.	
10/18/47	13,750	59			21	20			24 hr. conc. Neg.	
11/26/47		57		4	26	11	2			
10/20/47	Tuberculin patch test Negative									
10/21/47	Heterophile Antibody Negative									
10/23/47	Stool negative for ova and parasites									

The patient was given ascending doses of ragweed and house dust extracts; ten days after commencement of therapy there was definite improvement in that there was no need for the nightly aminophyllin suppositories the patient had been using. The use of the adrenalin spray was almost discontinued. On Oct. 8, 1947, he complained of a dry, irritating cough with some pain in the left upper chest, anteriorly. His temperature was 99° F. (oral). Examination of the chest was not unusual. A roentgenogram of the chest taken at this time was reported as follows: "There is a large consolidation beginning at the upper part of the left hilum and extending into the left upper chest and into the left apex. Two cavities are present; the highest is superimposed over the anterior part of the first rib. There is beginning infiltration into the right mid-lung field."

The temperature was normal three days later; the patient was ambulant and had no complaints. The cough had subsided. House dust and ragweed injections were continued, and both the asthma and hay fever symptoms were controlled.

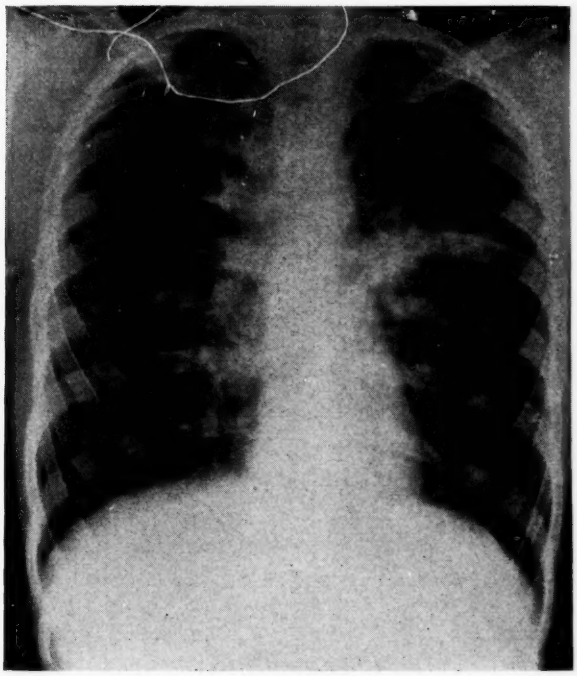

Fig. 1.—Oct. 8, 1947.

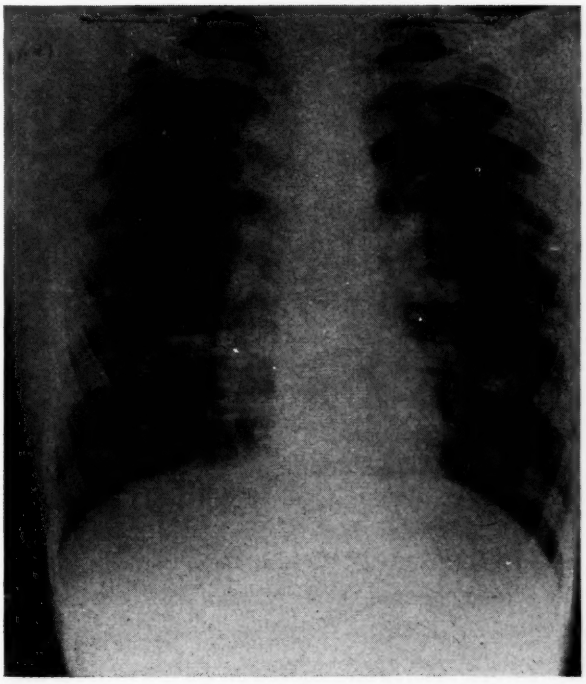

Fig. 2.—Oct. 25, 1947.

He was seen again on October 17, and examination of the chest revealed roughening of the inspiratory phase. He denied any wheezing. On October 25, the roentgenogram report read: "There is a marked decrease in the process in the left hilum and area adjacent to the left hilum with some increase in the left base below and lateral to the hilum; a mild and generalized increase in the right parahilar zone is present. The cavity superimposed over the free end of the left rib is still present, the other cavities below this area are no longer present."

The patient continued to gain weight and on Nov. 21, 1947, reached 102 pounds. He was sleeping well, but on exertion would have mild asthma which was controlled by the use of adrenalin spray. Report of a roentgenogram on Nov. 21, 1947, was: "Further clearing of the infiltrative process is noted in both lung fields. The previous markings in the left upper lobe which appeared to be cavitations, now resemble fibrous markings." The patient moved from the state in January, 1948; at that time he weighed 110 pounds, and his asthma was fairly well controlled.

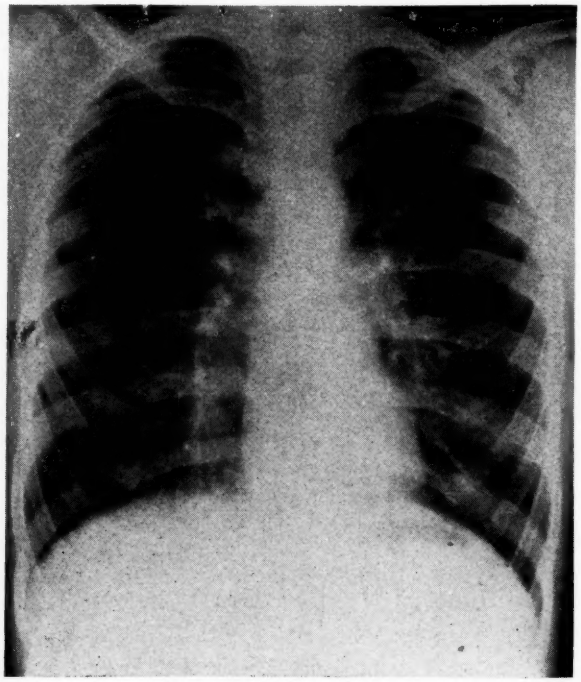

Fig. 3.—Nov. 21, 1947.

DISCUSSION

In the case reported, the diagnosis of an allergic reaction in the lung was entertained because of the transitory nature of the pulmonary infiltrations and the associated high blood eosinophilia in an allergic individual. It is interesting to speculate on the possibility of the specific pollen, ragweed, being the causative factor in this case. Meyer,[34] in 1937, reported the disappearance of nasal symptoms, eosinophilia, and pulmonary infiltrations in a case after removal from contact with a pollen.

It has been recently recognized that the parenchyma and interstitial tissue of the lung and pleural membrane may be the seat of an allergic response. Pulmonary involvement with radiological changes in angioedema was reported by

Vaughn and Hawke,[35] and by Feer.[36] Harkavy[4] has stated that bronchial spasms may be accompanied by inflammatory interstitial pulmonary changes with concomitant changes of the serous membranes as evidenced by pleural effusion and pericardial involvement.

The original concept of Loeffler's syndrome being a distinct disease entity is no longer tenable. Many of the recent case reports differ from the original description in that the pulmonary infiltrations persist for a longer time, there are recurrences and the outcome may be fatal. Loeffler's syndrome is but one phase of an allergic reaction of the lung tissue, a reaction which is reversible. This reaction may be similar to the immediate wheal-like reaction in which edema and swelling of the collagen fibrils occur. This type of reaction is usually mild in intensity and brief in duration with no permanent tissue damage resulting.

The type of tissue reaction that follows allergic insult, according to Bohrod,[37] depends upon such factors as the strength of the stimulus, the responsiveness of the host, and the velocity and duration of the reaction. The results of tissue injury may be anaphylactoid, necrotizing, or granulomatous. As noted in the section on pathology, the findings of an advanced organization of the pneumonic exudate, and the granulomatous character of the focal lesions are evidence only of a permanent, irreversible tissue reaction which although characteristic of allergy may not necessarily be of specific allergic origin. Similar histologic lesions have been described in rheumatic fever, periarteritis nodosa, scleroderma, and disseminated lupus erythematosus.

Tissue reactions in allergy are basically dependent upon an antigen-antibody reaction. Under certain circumstances, according to Walzer,[38] the shock tissue may even be stimulated nonspecifically. The degree of severity of the pulmonary involvement depends upon the factors mentioned along with the frequency of exposure and the resulting vascular damage. According to Harkavy,[39] the small vessels of the lung may in allergic states react as the shock organ. Increased permeability of the vessel walls results. The range of reactions may be of a transitory nature as seen in Loeffler's syndrome or of the more chronic, recurrent, and irreversible picture as reported by others. The evidence of preceding or related allergic manifestations such as urticaria, asthma, hay fever, migraine, and eczema in individuals exhibiting transitory pulmonary infiltrations more than suggests a definite relationship of such a pulmonary reaction with other allergic responses in individuals so predisposed.

The apparent cavitation in the lung field makes the case more striking. This is the second case reported in the literature. Elkeles and Butler[3] were the first to report such findings in a case of Loeffler's syndrome. The existence of emphysematous pleural blebs and bullae as the cause of certain thin-walled, rounded shadows in the lungs was proved by Arnell[2] and Doub.[40] Annular shadows in the lungs may be found in the bronchiectatic dilatation of the bronchi, localized pneumothoraces, varieties of cystic disease of the lung, breakdown of neoplastic tissue in pulmonary malignant tumors, and pyogenic infection of the lungs, as well as in pulmonary coccidioidomycoses.

Serial roentgenograms and observation of the clinical course in such cases presenting annular shadows in the pulmonary fields will help decide whether such shadows are true cavities or not.

The statistics on the coincidence of bronchial asthma and Loeffler's syndrome are meager, and from the reports quoted, the incidence ranges from 5 to 11 per cent. The reported incidence of asthma in the general population ranges from 3 to 4 per cent. In the series reviewed, asthma occurred in 69 per cent of the cases diagnosed as Loeffler's syndrome. This bears out the impression that in the asthmatic, one may expect a high percentage of incidence of transitory pulmonary infiltrations.

No single etiologic factor is responsible for the presence of transient pulmonary infiltrations. Such pulmonary findings occur more frequently in the allergic individual with bronchial asthma. That Loeffler's syndrome is an allergic phenomenon is accepted. Such a hypothesis may be based on histologic features, adequate clinical history, and observations relative to the presence of other associated allergic manifestations. The variability of response of the pulmonary tissue, both as to duration and final outcome as noted in many case reports, warrants a closer adherence in diagnosis to the criteria of Loeffler.

SUMMARY

1. A case of Loeffler's syndrome is presented in an allergic individual who had asthma and ragweed pollinosis.

2. Loeffler's syndrome is briefly reviewed, and is thought not to be a distinct entity, but rather one phase of an allergic pulmonary reaction.

3. Thirty-six cases of reported Loeffler's syndrome were reviewed; 69 per cent had a history of asthma either previous to or associated with the pulmonary findings. Only 25 per cent of the reported cases followed the diagnostic criteria of Loeffler's syndrome. Of the nine that were classified as Loeffler's syndrome, five (or 55 per cent) had asthma.

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VACCINIAL INFECTION IN CHILDREN WITH ATOPIC DERMATITIS*

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THE outbreak of a varicelliform eruption as a complication of infantile eczema during the mass vaccination campaign conducted in New York City in the spring of 1947 has already been described by Fries, Borne, and Barnes.[1] This communication deals with immunologic studies performed on sixteen of the twenty-one children in the borough of Brooklyn in whom the diagnosis of Kaposi's varicelliform eruption was made at that time; and the affinity of the vaccinia virus for the atopic eczematous skin is discussed.

Varicelliform eruptions complicating infantile eczema were given their classical description by Kaposi[2] in his discussion of herpetic lesions superimposed on the skin of ten eczematous patients. Although most subsequent publications carry detailed accounts of the clinical manifestations,[3-10] only a few of the more recent papers contain immunologic or viral studies[6, 7, 10-16] or evaluate the peculiar susceptibility of the atopic eczematous skin to certain viruses.

Briefly, the disease has the following characteristics. There is a preexistent allergic dermatitis, usually infantile eczema. The incubation period approximates ten days. The illness is ushered in by high fever, toxicity, and the appearance of many umbilicated pustules and vesicles from 3 to 8 mm. in size which are almost entirely confined to the already eczematized areas. Associated characteristics are lymphadenopathy and marked edema of the skin. The acute phase lasts about one week, and is followed by healing and convalescent phases, each also lasting approximately one week. An initial neutrophilic leucocytosis is usually followed by a lymphocytic leucopenia by the beginning of the second week. Frequent complications are bronchopneumonia, alopecia, and otitis media. Because it was felt that the previously reported mortalities were due to secondary invaders, penicillin in adequate amounts was administered parenterally and no mortalities occurred in our series.

ALLERGIC BACKGROUND

All of the sixteen children were allergic, judged by many criteria (involvement by an atopic dermatitis, family history, associated allergic manifestations, eosinophilia).

Inasmuch as the disease implants itself only on an "eczematous" skin and atopic dermatitis occurs most frequently in infancy, it is not surprising that eleven of these children were under two years of age.

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In all but two instances, the allergic rash was severe and generalized, involving face, neck, extremities, and trunk. Also, in all instances the past history of the development of the rash was characteristic of infantile allergic eczema, starting in the early months of infancy usually on the face and spreading to involve the remainder of the body.

Some evidences of specific food sensitivities were observed in ten of the children. Foods incriminated were egg, milk, wheat, and tomato. Associated asthma was observed in three children.

There was a history of allergy (that is, asthma, hay fever, or atopic dermatitis) in the immediate family (parents, siblings, or other immediate collaterals) of thirteen children (80.6 per cent).

Determinations of blood eosinophiles, made while the varicelliform eruption was in active progress, were consistently high, ranging from 10 to 52 per cent.

AFFINITY OF THE VACCINIAL VIRUS FOR THE ATOPIC ECZEMATOUS SKIN

From an analysis of our cases and of the 104 reported in the literature, this rash would seem to be peculiarly attracted to the atopic eczematous skin, although there have been rare instances reported of its occurrence in burns, impetigo, and scabies.[3, 17] It is indeed significant that only subjects with severe atopic dermatitis acquired this complication in the period of time immediately following the intensive vaccination program conducted in New York City in April, 1947, when 6,350,000 people were vaccinated against variola.[18] It would be unreasonable to suppose that persons with other skin diseases or burns did not come in contact with vaccinated individuals. In this city, entire hospital personnel caring for a vast variety of diseases and burns were vaccinated at this time and came in daily contact with their patients. Yet only those patients with severe and usually generalized atopic dermatitis contracted this exanthem. The affinity of the vaccinia virus for the allergic eczematous integument seems so strong as to open to question the possibility of the occurrence of Kaposi's varicelliform eruption in cutaneous diseases other than those due to atopy.

IMMUNOLOGIC STUDIES

The association of this small epidemic of a varicelliform eruption and the extensive use of the vaccinia virus at the time seemed to indicate the latter as a causative agent.

In order to demonstrate specific vaccinial antibodies induced by the disease, sera were drawn from the blood of nine patients, three to four weeks after the onset of their eruption. These patients had never been vaccinated. The convalescent sera were then neutralized in vitro with the specific pathogen, vaccinia virus. One serum was examined separately. Otherwise, two sera were pooled together.

The commercial glycerinated vaccinia virus* used in these experiments was potentiated by inoculating rabbit testicles serially ten times. Four days

*Sharp & Dohme, Inc., Philadelphia, Pa.

after the final inoculation, a 10 per cent emulsion of the testicular tissue was made in brain-heart broth.

Serial tenfold dilutions of the virus were then made and mixed with equal parts of the patients' sera; 0.1 ml. of the mixtures was injected intracutaneously into white rabbits. The results were read after four days (Table I). A control consisted of vaccinia virus in similar dilutions, but unmixed with convalescent sera.

All the sera showed definite evidences of vaccinial antibodies in terms of the protection afforded. The control in the dilutions 10:1 and 10:2 showed necrosis, while only erythema was observed with the test sera. Moreover, the highest dilution which produced a demonstrable reaction was 10:4 in the control, while in sera 2, 3, and 5 it was 10:3; and in sera 1 and 4 it was 10:2.

TABLE I. TITRATION OF PROTECTIVE ANTIBODIES AGAINST VACCINIA VIRUS

VIRUS DILUTION	SERUM 1 J. Z. & M. H.	SERUM 2 M. S. & D. A.	SERUM 3 R. S. & R. E.	SERUM 4 V. S. & D. H.	SERUM 5 F. W.	CONTROL
10:1	E +++	E +++	E +++	E ++++	E +++	N
10:2	E ++	E +	E +++	E ++	E +++	N
10:3	0	E +	E ++	E ±	E ++	E ++++
10:4	0	E ±	E ±	0	0	E +
10:5	0	0	0	0	0	E ±
10:6	0	0	0	0	0	0
10:7	0	0	0	0	0	0

E, Erythema; 0 to ++++ denoting degree of reaction. N, Necrosis.

To obtain further proof of the presence of vaccinial antibodies, we attempted to vaccinate fifteen of our patients during convalescence. These children had never been vaccinated prior to our "challenge" inoculation. Multiple puncture and scratch methods were used on alternate patients with a vaccinia virus known to produce "takes" in susceptible individuals. All fifteen reacted with an immune response, consisting of a small papule which appeared within twenty-four to forty-eight hours, and then receded rapidly.

TABLE II. COMPARISON OF GENERALIZED VACCINIA AND KAPOSI'S VARICELLIFORM ERUPTION DUE TO VACCINIA VIRUS

CHARACTERISTICS	GENERALIZED VACCINIA	KAPOSI'S VARICELLIFORM ERUPTION
Exanthem	A few disseminated, relatively large lesions, or a large pustule surrounded by "satellite" vesicles; scarring is usually permanent	Crops of closely packed umbilicated vesicles and pustules averaging only 5 mm. in diameter; scarring impermanent
Dermatropism	Lesions do not show any predilection for any type of skin or particular site	Superimposed exanthem shows definite preference for atopic eczematoid areas, especially those on the face
Allergic background	Pre-existent dermatoses infrequent; family history negative for atopy	Atopic dermatitis always present; family history usually positive
Systemic reactions	Usually slight or absent; if present, of short duration	Fever and toxemia almost always present, of 5 to 7 days' duration
Contagion	Usually contracted by autoinoculation, occasionally by external contact	Most frequently from external source
Incubation period	2 to 10 days	5 to 19 days
Complications	Occasionally cellulitis, lymphadenitis, and conjunctivitis	Frequently bronchopneumonia, alopecia, otitis media

CLASSIFICATION

Disseminated vaccinial infections of the skin would seem to fall into two broad classifications: those occurring on normal skin, and those occurring as a complication of pre-existing dermatitis.

In order to illustrate the differences between these two categories, we reviewed the histories of ten hospital admissions for "generalized vaccinia" occurring on normal intact skin, and compared them to the sixteen cases of Kaposi's varicelliform eruption under discussion. Although the numbers involved were few, it was apparent that certain differences existed between the two. These differences, cutaneous and systemic, were so consistent and characteristic as to warrant this twofold classification (Table II).

DISCUSSION

The affinity of this virus[19, 20] for the allergic "eczematous" skin is a striking feature in our series. However, since there is little known on this subject, any of the following speculations might be valid. (1) Since the virus is disseminated by the general circulation, damaged skin (such as the chronic eczemoid skin) might favor localization of the vaccinia virus, as shown in animal experiments by Calmette.[21] (2) The weeping eczematous lesions might provide a particularly favorable culture medium for the vaccinia virus as does the chorio-allantoic membrane of the chick embryo. This is supported by our observation that the spread of the rash is contiguous with the already damaged areas and stops at the point where intact skin appears. (3) That the damaged "eczematous" skin offers open sites for entry of the vaccinia virus does not seem a valid explanation because other skin diseases offer lesions through which the virus would have equally easy access. (4) If an analogy can be drawn from animal experiments, dermatoses of allergic origin may have a potentiating effect on the vaccinia virus perhaps by the release of a histamine-like substance.[22] (5) It is possible that the susceptibility of the host to the vaccinia virus is an inheritable predisposition determined by genetic factors, as is the allergic constitution. If this assumption were correct, the parallel susceptibility in the same individual would suggest chromosomal linkage.

Nomenclature.—The term "Kaposi's varicelliform eruption," originally used to describe a "varicelliform" lesion on the eczematous skin, has since been described variously by a large number of designations: pustulosis vacciniformis acuta (Freund[23]), pustulosis acuta varioliformis (Juliusberg[24]), dermatitis vaccinia (Ronchese[9]), generalized vaccinia (Mustard and Hendrick[25])—to mention a few.

Although this eruption usually has been termed "varicelliform," the exanthem in actuality more closely resembles variola than varicella. The lesions are monomorphous and predominantly umbilicated (Fig. 1, 2, 3) in contrast to those of varicella. The severe systemic symptoms also compare with those of variola.

Fig. 1.

Fig. 2.

Fig. 1.—First stage. Group of early vesicular lesions.
Fig. 2.—Second stage. Cluster of umbilicated pustular lesions.

Fig. 3.—Third stage. Cluster of crusted lesions.

Sulzberger,[26] in 1942, intimated that certain cases of Kaposi's varicelliform eruption were in all probability due to generalized cutaneous and systemic infection with herpes simplex virus superimposed on an atopic dermatitis. Since then, several observers have verified this impression.[6, 10, 11, 13, 16] Other writers have identified the vaccinia virus as playing a similar role in the etiology of this disease.[7, 25] As the number of viral studies increase and methods of identification improve, it is probable that other organisms may be incriminated.

It has already been stated that this clinical syndrome should be considered a specific entity.[27] In view of the existence of a definite virus etiology

and of a specific rash appearing on the atopic "eczematous" skin, we maintain that this illness is a specific exanthem. Inasmuch as it has a determinate incubation period, a typical self-limited course, and sequelae, it should be catalogued among the contagious diseases as are other exanthemas.

We feel that the term "Kaposi's varicelliform eruption" should be preserved until a standardized international nomenclature can be found. This would avoid a profusion of terminology for an already well-defined clinical entity. However, in those instances in which a specific virus is identified, a modifying phrase, "due to herpes" or "due to vaccinia," might be appended.

Anergy.—We noted during the acute febrile phase of the varicelliform eruption a marked but temporary clearance of the eczematous involvement in some of our subjects. We have also observed this temporary improvement of the chronically eczematized skin during the febrile phases of such acute infectious diseases as measles, upper respiratory infections, and pneumonia. The phenomenon has been commented upon by others.[28]

Therapy.—Several forms of local, enteral, and parenteral therapy have been suggested. However, we believe that this disease itself runs a self-limited, though stormy, course and that the formerly reported high mortality rate was caused by complications. All of our sixteen patients recovered due, we believe, to the control of secondary invaders by intramuscular injection of 400,000 units of penicillin daily. Specific convalescent human serum and irradiation, although their use was unnecessary in our patients, also would seem to be reasonable therapeutic adjuvants.[29*]

SUMMARY

1. By neutralization and vaccination experiments, vaccinia virus was proved to be the etiological agent in a recent outbreak of a varicelliform eruption resembling that described by Kaposi.

2. The atopic eczematoid skin is uniquely susceptible to virus invasion, particularly by the vaccinia virus.

3. The varicelliform eruption of Kaposi, when due to vaccinia virus, and generalized vaccinia are different clinical entities.

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*Dr. Marion B. Sulzberger in a personal communication reported that Drs. R. L. Baer and Otis Miller of his Department have recently demonstrated the effectiveness of local and systemic administrations of aureomycin in two severe cases of Kaposi's varicelliform eruption presumably due to herpes simplex virus.

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